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(54) Title: 28 HUMAN SECRETED PROTEINS

(57) Abstract: The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

28 Human Secreted Proteins

Field of the Invention

This invention relates to newly identified polynucleotides, polypeptides encoded by these polynucleotides, antibodies that bind these polypeptides, uses of such polynucleotides, polypeptides, and antibodies, and their production.

Background of the Invention

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoeitin. Thus, in light of

the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

Detailed Description

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As

shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched , for example, as a result of

ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Polynucleotides and Polypeptides of the Invention

FEATURES OF PROTEIN ENCODED BY GENE NO: 1

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with mouse and human fibulin proteins (see, e.g., Genbank accession numbers emb|CAA37772.1| and pir|S78040|S78040, all references available through these accession numbers are hereby incorporated by reference herein), which may play a role as connecting elements in the extracellular matrix. Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: CNPGFRLVGPSSVVCLPNGTWTGEQPHCRGISECSSQPCQNGGTCVEGVNQY RCICPPGRTGNRCQHQAQTAAPEGSVAGDSAFSRAPRCAQVERAQHCSCEAG FHLSGAAGDSVCQDVNECELYGQEGRPRLCMHACVNTPGSYRCTCPGGYRT LADGKSCEDVDECVGLQPVCPQGTTCINTGGSFQCVSPECPEGSGNVSYVKT SPFQCERNPCPMDSRPCRHLPKTISFHYLSLPSNLKTPITLFRMXTASAPGRAG PNSLRFGIVGGNSRGHFVMQRSDRQTGDLILVQNLEGPQTLEVDVDMSEYLD RSFQANHVSKVTIFVS (SEQ ID NO: 172). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

This gene is expressed primarily in the following tissues/cDNA libraries: Human Placenta and to a lesser extent in Activated T-Cell (12hs)/Thiouridine labelledEco; Soares placenta Nb2HP; Placenta; Human adult (K.Okubo); Human Whole Brain #2 - Oligo dT > 1.5Kb; Stratagene neuroepithelium (#937231); NCI_CGAP_Pr28; Colon Normal II; Anergic T-cell; T Cell helper I; Soares ovary tumor NbHOT; Osteoblasts; Soares melanocyte 2NbHM and Soares_NFL_T_GBC_S1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the immune system, vascular diseases, cancers, and/or diseases associated with disorders involving extracellular matrix proteins. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and/or vascular systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, vascular, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The homology of this protein to fibulin, a secreted glycoprotein, indicates a role in the synthesis and turnover of the fibrillar extracellular matrix. The elevated level of expression of this protein in T-cells indicates a role for this matrix remodelling during inflammation and in arthritis, asthma, immune deficiency diseases such as AIDS, and leukemia; in the treatment/detection of thymus disorders such as Graves Disease, lymphocytic thyroiditis, hyperthyroidism and hypothyroidism. Its elevated level of expression in a number of cancers including ovarian and prostate carcinomas indicates a role for the protein in mediating tumor metastasis. The protein would be useful in the detection, treatment, and/or prevention of a variety of vascular disorders and conditions, which include, but are not limited to miscrovascular disease,

vascular leak syndrome, aneurysm, stroke, embolism, thrombosis, coronary artery disease, arteriosclerosis, and/or atherosclerosis. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 2

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: HELQDTVALDHGGCCPALSRLVPRGFGTEMWTLFALSGPLFLFQVLTFMIYIV STVFCGHLGKVELASVTLAVAFVNVCGVSVGVGLSSACDTLMSQSFGSPNK KHVGVILQRGALVLLLCCLPCWALFLNTQHILLLFRQDPDVSRLTQDYVMIFI PGLPVIFLYNLLAKYLQNQVQVFECVGRPFSQHTALFQWEGGLGLSPSLHHL (SEQ ID NO: 173), FGTEMWTLFALSGPLFLFQVLTFMIYIVSTVFCGHLGKVELASVTLAVAFVNV CGVSVGVGLSSACDTLMSQSFGSPNKKHVGVILQRGALVLLLCCLPCWALFL NTQHILLLFRQDPDVSRLTQDYVMIFIPGLPVIFLYNLLAKYLQNQ (SEQ ID NO: 174), and QVLSGVVGNCVNGVANYALVSVLNLGVRGSAYANIISQFAQTVFLLLYIVLK KLHLETWAGWSSQCLQDWGPFFSLAVPSMLMICVEWWAYEIGSFLMGLLSV VDLSAQAVIYEVATVTYMV (SEQ ID NO: 175). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in the following tissues/cDNA libraries: Epididiymus and Kidney

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: male reproductive and renal disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the renal and male reproductive systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, renal, cancerous and wounded tissues) or bodily fluids (e.g., semen, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in epididymus indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, detection, prevention, and/or treatment of male infertility and/or associated disorders of the male reproductive system. In addition, the tissue distribution in kidney indicates that polynucleotides and polypeptides corresponding to this gene would be useful in the treatment, prevention, diagnosis, and/or detection of kidney diseases including renal failure, nephritus, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 3

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: GDTGEIKSEVREQINAKVAEWREEGKAEIIPGV (SEQ ID NO: 176), and VLFIDEVHMLDIESFSFLNRALESDMAPVLIMATNRGITR (SEQ ID NO: 177). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in colon tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the gastrointestinal tract, including but not limited to diseases of the colon and/or immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and/or gastrointestinal systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, colon, immune, cancerous and wounded tissues) or bodily fluids (e.g., feces, bile, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in colon tissue indicates the gene or its products would be useful for the diagnosis, treatment and/or prevention of disorders of the colon,

including inflammatory disorders such as, diverticular colon disease (DCD), inflammatory colonic disease, Crohn's disease (CD), non-inflammatory bowel disease (non-IBD) colonic inflammation; ulcerative disorders such as, ulcerative colitis (UC), amebic colitis, eosinophilic colitis; noncancerous tumors, such as, polyps in the colon, adenomas, leiomyomas, lipomas, and angiomas. In addition, polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, treatment and/or detection of tumors, especially of the intestine, such as, carcinoid tumors, lymphomas, cancer of the colon and cancer of the rectum, as well as cancers in other tissues where expression has been indicated. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 4

The gene encoding the disclosed cDNA is believed to reside on chromosome 19. Accordingly, polynucleotides related to this invention would be useful as a marker in linkage analysis for chromosome 19.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 322 to 338 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 339 to 403 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in the following tissues/cDNA libraries: Colon Normal II and to a lesser extent in Human Osteoarthritic Cartilage Fraction IV; Human Osteoarthritic Cartilage Fraction III; Smooth muscle, control, re-excision; Stromal cells 3.88; Human Thymus Stromal Cells; NTERA2, control; Fetal Heart; human tonsils and Osteoblasts.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the skeletal and/or immune systems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skeletal and immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., bone, skeletal, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

This protein shows elevated levels of expression in osteoblasts indicating a role in bone differentiation, in particular osteoporosis as well as disorders afflicting connective tissues (e.g., arthritis, trauma, tendonitis, chrondomalacia and inflammation. Its expression is also elevated in both tonsils and thymus indicating a role in immune modulation and as such would be useful for the treatment, prevention, diagnosis, or detection of immune or hematopoietic disorders including arthritis, asthma, immunodeficiency diseases and leukemia. Similarly, protein would also be useful in the diagnosis or treatment of various autoimmune disorders (i.e., rheumatoid arthritis, lupus, scleroderma, and dermatomyositis), dwarfism, spinal deformation, joint abnormalities, and chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid, etc.). Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 5

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with yeast proteins similar to glucosamine-6-sulfatase (see, e.g., Genbank accession number gb|AAA83618.1| (or sequence shown in SEQ ID NO:178), all information available through the recited accession number is incorporated herein by reference). Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

NIILVLTDDQDVELGSMQVMNKTRRIMEQGGAHFINAFVTTPMCCPSR SSILTGKYVHNHNTYTNNENCSSPSWQAQHESRTFAVYLNSTGYRTAFFGKY LNEYNGSYVPPGWKEWVGLLKNSRFYNYTLCRNGVKEKHGSDYSKDYLTD LITNDSVSFFRTSKKMYPHRPVLMVISHAAPHGPEDSAPQYSRLFPNASQHITP SYNYAPNPDKHWIMRYTGPMKPIHMEFTNMLQRKRLQTLMSVDDSMETIYN MLVETGELDNTYIVYTADHGYHIGQFGLVKGKSMPYEFDIRVPFYVRGPNVE AGCLNPHIVLNIDLAPTILDIAGLDIPADMDGKSILKLLDTERPVNRFHLKKKM RVWRDSFLVERGKLLHKRDNDKVDAQEENFLPKYQRVKDLCQRAEYQTAC EQLGQKWQCVEDATGKLKLHKCK (SEQ ID NO: 179). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 5 to 21 of the amino acid sequence referenced in

Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 22 to 870 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

This gene is expressed primarily in the following tissues/cDNA libraries: Primary Dendritic Cells, lib 1 and to a lesser extent in Human Pancreas Tumor; Human MCF7 cDNA subtracted with MDA-MB-231 cDNA; Human Pancreas Tumor, Reexcision; Hodgkin's Lymphoma II; Human 8 Week Whole Embryo; Human 8 Week Whole Embryo, subtracted; Human Ovary; CD34 depleted Buffy Coat (Cord Blood), re-excision; Soares_placenta_8to9weeks_2NbHP8to9W; Human Endometrial Tumor; Soares_pregnant_uterus_NbHPU; Soares_NhHMPu_S1; pBMC stimulated w/ poly I/C; Glioblastoma; Stratagene NT2 neuronal precursor 937230; Stratagene hNT neuron (#937233); Neutrophils IL-1 and LPS induced; Primary Dendritic cells,frac 2; Human Fetal Heart; Endothelial-induced; Endothelial cells-control; NCI_CGAP_Brn23; Keratinocyte; Nine Week Old Early Stage Human; Colon Tumor II; Soares_fetal_liver_spleen_1NFLS_S1; HCC cell line metastisis to liver; Human Testes; NCI_CGAP_Mel3; Larynx tumor; Human Tongue, frac 2; Schiller oligodendroglioma; Testes; Human colorectal cancer; Schiller meningioma; NCI_CGAP_Brn35; NCI_CGAP_Eso2; HUMAN STOMACH; Aorta endothelial cells + TNF-a; Lung Carcinoma A549 TNFalpha activated; NCI_CGAP_Ut4; Hepatocellular Tumor; Human adult (K.Okubo); H Female Bladder, Adult; Morton Fetal Cochlea; wilm's tumor; Healing groin wound, 6.5 hours post incision; Human Manic Depression Tissue; H. Lymph node breast Cancer; NCI_CGAP_Alv1; Human Chronic Synovitis; NCI_CGAP_Ut2; NCI_CGAP_Ut1; Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma; human ovarian cancer; NCI_CGAP_Gas4; Human Hypothalmus,Schizophrenia; Olfactory epithelium,nasalcavity; Human Chondrosarcoma; Macrophage (GM-CSF treated); Soares adult brain N2b5HB55Y; Macrophage-oxLDL, re-excision; NCI_CGAP_Pan1; NCI_CGAP_Co3; Colon Tumor; Stratagene colon (#937204); Colon Normal II; NCI_CGAP_Co8; Dendritic cells, pooled; Pancreas normal PCA4 No; NCI_CGAP_Kid5; HUMAN B CELL LYMPHOMA; Human Bone Marrow, treated; NCI_CGAP_Lu5; neutrophils control; Human Cerebellum;

Soares_fetal_heart_NbHH19W; Soares infant brain 1NIB and Soares fetal liver spleen 1NFLS.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the immune system such as cancer, and diseases associated with proliferative conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, proliferating, pancreas, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression in primary dendritic cells indicates that polynucleotides and polypeptides corresponding to this gene would be useful for prevention, diagnosis and/or treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes indicates a usefulness in the treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and

graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders; cancer, especially cancer in the tissues where expression has been indicated; and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Polypeptides and polynucleotides of the invention are useful for the treatment, detection, and/or prevention of the following non-limiting and exemplary diseases

and/or disorders: pancreatic tumor, Hodgkin's lymphoma ii; endometrial tumor; larynx tumor; schiller oligodendroglioma; colorectal cancer; schiller meningioma; lung carcinoma, hepatocellular tumor; manic depression, breast cancer; carcinoma; chondrosarcoma; and colon tumor.

FEATURES OF PROTEIN ENCODED BY GENE NO: 6

This gene is expressed primarily in the following tissues/cDNA libraries: Neutrophils IL-1 and LPS induced.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the immune system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neutrophils indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes indicates a usefulness in the treatment of cancer (e.g., by boosting immune

responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 7

When tested against T cell lines, supernatants removed from cells containing this gene activated the IL-2 promoter element. Thus, it is likely that this gene activates T cells. The gene encoding the disclosed cDNA is believed to reside on chromosome 14. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 14.

This gene is expressed primarily in the following tissues/cDNA libraries: normalized infant brain cDNA and to a lesser extent in Soares_NhHMPu_S1; Soares infant brain 1NIB; Soares melanocyte 2NbHM; Soares_multiple_sclerosis_2NbHMSP; NCI_CGAP_Lu5; Soares placenta Nb2HP;

Human 8 Week Whole Embryo; NCI_CGAP_GCB1; Human adult small intestine,re-excision; Monocyte activated, re-excision; Human T-Cell Lymphoma; Human Eosinophils; 12 Week Early Stage Human II, Reexcision; Human Fetal Lung; Human Adult Liver, subtracted; Human promyelocyte; Lung Carcinoma A549 TNFalpha activated; Hepatocellular Tumor,re-excision; Synovial Fibroblasts (Il1/TNF), subt; NCI_CGAP_Lym12; NCI_CGAP_Pr3; NCI_CGAP_Ut1; 12 Week Old Early Stage Human, II; Human Adipose; Human Activated T-Cells, re-excision; Soares breast 2NbHBst; NCI_CGAP_Pan1; 12 Week Old Early Stage Human; Human Substantia Nigra; Smooth muscle, serum treated; Brain frontal cortex; Soares breast 3NbHBst; Human Fetal Heart; Endothelial cells-control; Human Osteoclastoma; Human Amygdala; Soares_placenta_8to9weeks_2NbHP8to9W; Soares_pregnant_uterus_NbHPU; Soares_NFL_T_GBC_S1 and Soares fetal liver spleen 1NFLS.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: developmental diseases and disorders, cancer, and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and/or immune systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., fetal, immune, proliferating, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Moreover, the expression within embryonic tissue and other cellular sources marked by proliferating cells, and the activity in an IL-2 assay indicates that polynucleotides and polypeptides corresponding to this gene would be useful in the regulation of cellular division, and in the diagnosis, detection, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative

conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention would be useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Polynucleotides and polypeptides of the present invention may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. In addition, the primary tissue distribution in brain indicates that polynucleotides and polypeptides corresponding to this gene would also be useful for the diagnosis and treatment of neurological and/or behavioral disorders including Alzheimers Disease, Parkinson's Disease, Huntington's Disease, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder and panic disorder.

FEATURES OF PROTEIN ENCODED BY GENE NO: 8

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MTSGPGGPAAAAGGRKENHQWYVCNREKLCESLQAVFVQSYLDQGT
QIFLNNSIEKSGWLFIQLYHSFVSSVFSLFMSRTSINGLLGRGSMFVFSPDQFQR
LLKINPDWKTHRLLDLGAGDGEVTKIMSPHFEEIYATELSETMIWQLQKKKY
RVLGINEWQNTGFQYDVISCLNLLDRCDQPLTLLKDIRSVLEPTRGRVILALV

LPFHPYVENVGGKWEKPSEILEIKGQNWEEQVNSLPEVFRKAGFVIEAFTRLP YLCEGDMYNDYYVLDDAVFVLKPV (SEQ ID NO: 180). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA maps to the 16p11.2 cytologic band. Accordingly, polynucleotides related to this invention would be useful as a marker in linkage analysis for chromosome 16, and more specifically this band.

This gene is expressed primarily in the following tissues/cDNA libraries: Soares melanocyte 2NbHM and to a lesser extent in Glioblastoma; Larynx carcinoma III; Soares_parathyroid_tumor_NbHPA; Soares_total_fetus_Nb2HF8_9w; Soares fetal liver spleen 1NFLS; Soares_senescent_fibroblasts_NbHSF; Mammary Gland; NCI_CGAP_Br2; NCI_CGAP_Co8; NCI_CGAP_Brn23; Soares ovary tumor NbHOT; NCI_CGAP_Lu5; NCI_CGAP_Kid3; Soares_placenta_8to9weeks_2NbHP8to9W; Soares_fetal_lung_NbHL19W; Soares placenta Nb2HP; NCI_CGAP_GCB1; NCI_CGAP_Br3; Smooth Muscle- HASTE normalized; Ovary, Cancer (15395): Grade II Papillary Carcinoma (1935AIF); Hippocampus, Alzheimer Subtracted; Monocyte activated, re-excision; Stratagene HeLa cell s3 937216; NCI_CGAP_CLL1; Colon Tumor; Monocyte activated; Soares_fetal_liver_spleen_1NFLS_S1; Soares_fetal_heart_NbHH19W; Soares_NhHMPu_S1; Soares infant brain 1NIB; Smooth muscle-ILb induced; Synovial hypoxia; Spleen metastic melanoma; Human Umbilical Vein, Reexcision; Human Testes Tumor, re-excision; NCI_CGAP_Co3; Colon Carcinoma; NCI_CGAP_Kid11; Dendritic cells, pooled; Human Fetal Lung III; Human Placenta; Human Adult Pulmonary,re-excision; Endothelial cells-control; NCI_CGAP_Kid5; Bone Marrow Cell Line (RS4,11); normalized infant brain cDNA; Keratinocyte; T cell helper II; Soares_pregnant_uterus_NbHPU; Soares_testis_NHT; Human Pre-Differentiated Adipocytes; Saos2 Cells, Vitamin D3 Treated; Soares_NbHFB;

stromal cell clone 2.5; Human Adult Liver, subtracted; NCI_CGAP_Eso2; NCI_CGAP_Lu24; Normal Human Trabecular Bone Cells; H. Atrophic Endometrium; Supt Cells, cyclohexamide treated; H. Epididiymus, caput & corpus; Early Stage Human Lung, subtracted; Human Soleus; Human Pineal Gland; NCI_CGAP_GC3; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Human Tonsils, Lib 2; Dendritic Cells From CD34 Cells; Healing groin wound - zero hr post-incision (control); HEL cell line; Human Synovium; STROMAL - OSTEOCLASTOMA; Human adult (K.Okubo); human corpus colosum; NCI_CGAP_Co9; Synovial hypoxia-RSF subtracted; Human endometrial stromal cells-treated with progesterone; Soares adult brain N2b4HB55Y; NCI_CGAP_Lym12; Healing groin wound, 6.5 hours post incision; Human Prostate; T-Cell PHA 16 hrs; TF-1 Cell Line GM-CSF Treated; HM1; NCI_CGAP_Kid6; human ovarian cancer; Stromal cell TF274; Macrophage-oxLDL; Liver, Hepatoma; Human Hippocampus; Spinal cord; Human Activated T-Cells, re-excision; Epithelial-TNFa and INF induced; Soares adult brain N2b5HB55Y; NTERA2, control; Hepatocellular Tumor, re-excision; Macrophage-oxLDL, re-excision; CHME Cell Line,untreated; 12 Week Old Early Stage Human; Human Substantia Nigra; Smooth muscle, serum treated; Colon Normal II; Soares breast 3NbHBst; Normal colon; Human Fetal Kidney, Reexcision; Human Testes, Reexcision; 12 Week Early Stage Human II, Reexcision; Human Fetal Heart; Endothelial-induced; NCI_CGAP_Brn25; Smooth muscle,control; Human Bone Marrow, treated; Human fetal heart, Lambda ZAP Express; HM3; Human Testes; H. Frontal cortex,epileptic,re-excision; Activated T-cell(12h)/Thiouridine-re-excision; Nine Week Old Early Stage Human; Colon Normal III and Soares_NFL_T_GBC_S1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: developmental diseases and disorders, cancer, and other disorders related to proliferative cells and tissues. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, reproductive, developmental

systems and/or skin, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skin, proliferating cells or tissues, fetal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that polynucleotides and polypeptides corresponding to this gene would be useful in regulating cellular division, and in the diagnosis, detection, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. The primary tissue distribution in melanocytes indicates that the protein product of this clone is useful for the diagnosis and treatment of skin disorders including melanocytoma's. Furthermore, the protein may also be used to determine biological

activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 9

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: PAHLATTSRWNPATICEMGHDAVQWRVRAGVSPVSTTFVTDVLSERR SLPSLTCLKRPEPESALAVSLRPAPGGASLLPRWGRFPGPRGLRCRLPLHRTVL SFPHPPSEAPAYSRGVNKQMEAEG (SEQ ID NO: 181) and LPSLTCLKRPEPESALAVSLRPAPGGASLLPRWGRFPGPRGLRCRLPLHRTVLS FPHPPSEAPAY (SEQ ID NO: 182). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Salivary Gland, Lib 2.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: digestive system disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., salivary, digestive, cancerous and wounded tissues) or bodily fluids

(e.g., saliva, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression in salivary gland tissue indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the treatment, detection, and/or prevention of digestive diseases and/or disorders which include, but are not limited to, amylase and/or secretory disorders. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 10

This gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1NIB and to a lesser extent in Soares_testis_NHT; normalized infant brain cDNA; Human 8 Week Whole Embryo; Nine Week Old Early Stage Human; Soares_fetal_lung_NbHL19W; Soares fetal liver spleen 1NFLS; Normalized infant brain, Bento Soares; Human Umbilical Vein Endothelial Cells, fract. A; Frontal lobe,dementia,re-excision; Early Stage Human Lung, subtracted; Human Prostate Cancer, Stage C fraction; Human Umbilical Vein, Reexcision; Temporal cortex-Alzheizmer, subtracted; Human Ovary; Pancreas Islet Cell Tumor; Human fetal heart, Lambda ZAP Express; NCI_CGAP_Lu5; T cell helper II; Soares_NFL_T_GBC_S1 and Soares_NhHMPu_S1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the central nervous system, developmental disorders and/or disorders associated with proliferating cells. Similarly, polypeptides and

antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, proliferating cells or tissues, and/or developmental tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., central nervous system, neural, neuronal, developmental, fetal, proliferating tissues, including but not limited to cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that the polynucleotides and polypeptides corresponding to this gene would be useful in regulation of cellular division, and in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be

used to gain new insight into the regulation of cellular growth and proliferation. In addition, the tissue distribution in infant brain and other central nervous system tissues indicates the protein product of this clone would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 11

The gene encoding the disclosed cDNA is believed to reside on chromosome 10. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 10.

This gene is expressed primarily in the following tissues/cDNA libraries: Hepatocellular Tumor, re-excision; Soares infant brain 1NIB and to a lesser extent in

b4HB3MA Cot8-HAP-Ft; NCI_CGAP_Kid11; NCI_CGAP_Kid3; normalized infant brain cDNA; Soares fetal liver spleen 1NFLS; H. Normalized Fetal Liver, II; Liver HepG2 cell line.; Hepatocellular Tumor; Synovial hypoxia-RSF subtracted; NCI_CGAP_Ut1; Colon Carcinoma; Human Testes, Reexcision; Human fetal heart, Lambda ZAP Express; NCI_CGAP_Lu5; Soares_total_fetus_Nb2HF8_9w; Colon Normal III and Soares_fetal_liver_spleen_1NFLS_S1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders associated with proliferating cells, such as cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of proliferating cells, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., fetal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The expression within embryonic tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues -

particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 12

This gene is expressed primarily in the following tissues/cDNA libraries: NCI_CGAP_GCB1 and to a lesser extent in Human fetal heart, Lambda ZAP Express; Human 8 Week Whole Embryo; Soares placenta Nb2HP; Soares infant brain 1NIB; Soares fetal liver spleen 1NFLS; Synovial Fibroblasts (Il1/TNF), subt; NCI_CGAP_Brn23; Primary Dendritic Cells, lib 1; NCI_CGAP_Thy1; 12 Week Old Early Stage Human, II; Human Adipose; Stratagene hNT neuron (#937233); Rejected Kidney, lib 4; Soares_senescent_fibroblasts_NbHSF; Soares_parathyroid_tumor_NbHPA; Soares_fetal_heart_NbHH19W; Soares_NhHMPu_S1; Larynx Tumour; Apoptotic T-cell; Stratagene fetal spleen (#937205); Human Thymus Stromal Cells; 12 Week Old Early Stage Human; Human

Placenta; Pancreas normal PCA4 No; Human Adult Pulmonary,re-excision; Smooth muscle,control; Soares ovary tumor NbHOT; Bone Marrow Cell Line (RS4,11); Nine Week Old Early Stage Human; Soares_fetal_lung_NbHL19W; Soares_total_fetus_Nb2HF8_9w; Hypothalamus; Prostate; Human aorta polyA+ (TFujiwara); Human fetal lung; Frontal Lobe, Dementia; Human Adult Spleen; NCI_CGAP_Br1.1; Smooth Muscle Serum Treated, Norm; Human Lung; Human adult small intestine,re-excision; Ovary, Cancer: (4004332 A2); HEL cell line; Human endometrial stromal cells-treated with estradiol; STROMAL - OSTEOCLASTOMA; Hepatocellular Tumor,re-excision; Synovial IL-1/TNF stimulated; Human adult (K.Okubo); H Female Bladder, Adult; Human Umbilical Vein, Endo. remake; Stratagene ovary (#937217); Human Stomach,re-excision; NCI_CGAP_Co10; Human Manic Depression Tissue; Brain Frontal Cortex, re-excision; NCI_CGAP_Alv1; Human Chronic Synovitis; NCI_CGAP_Ut2; Stratagene lung carcinoma 937218; Human Prostate; Stratagene NT2 neuronal precursor 937230; Monocyte activated, re-excision; Clontech human aorta polyA+ mRNA (#6572); T-Cell PHA 24 hrs; Human Pancreas Tumor, Reexcision; Human Placenta (re-excision); NCI_CGAP_Br2; Human Chondrosarcoma; Macrophage (GM-CSF treated); Hepatocellular Tumor, re-excision; Human Gall Bladder; PC3 Prostate cell line; Colon Tumor; Colon Carcinoma; Brain frontal cortex; Normal colon; B-cells (stimulated); Human Ovarian Cancer Reexcision; 12 Week Early Stage Human II, Reexcision; human tonsils; NCI_CGAP_GC6; Human Osteoclastoma; Anergic T-cell; Human Amygdala; Stratagene lung (#937210); Human Bone Marrow, treated; Human Endometrial Tumor; Hodgkin's Lymphoma II; NCI_CGAP_Lu5; Osteoblasts; Soares melanocyte 2NbHM; Soares_pregnant_uterus_NbHPU; Soares_fetal_liver_spleen_1NFLS_S1 and Soares_NFL_T_GBC_S1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the immune system, and/or central nervous system and/or developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above

tissues or cells, particularly of the immune system, central nervous system, and/or developmental tissues, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, neuronal, fetal, developmental, cancerous and wounded tissues) or bodily fluids (e.g., amniotic fluid, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in immune tissues, such as germinal B cells, indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the detection, treatment, and/or prevention of immune system disorders Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes indicates a usefulness in the treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell

types. In addition, the tissue distribution in central nervous system tissues indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 13

The gene encoding the disclosed cDNA is believed to reside on chromosome 15. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 15.

This gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1NIB and to a lesser extent in NCI_CGAP_GCB1; Soares adult brain N2b5HB55Y; Normal Ovary, Premenopausal; CD34 positive cells (cord

blood),re-ex; Synovial IL-1/TNF stimulated; Monocyte activated, re-excision; Human Activated Monocytes; CHME Cell Line,treated 5 hrs; Human Substantia Nigra; Primary Dendritic cells,frac 2; Human Amygdala; CD34 positive cells (Cord Blood); H. Frontal cortex,epileptic,re-excision; normalized infant brain cDNA and Human 8 Week Whole Embryo.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the central nervous system or immune system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system or immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, central nervous system, neural, neuronal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in central nervous system tissues indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and

perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. In addition, the tissue distribution in immune tissues, indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes indicates a usefulness in the treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 14

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence: MIGLGIGCAGQRDQAPPYLAPPSQEPGDAAKAVNRGGGTVGAAGSRGWGET CGHVASMAPACQILRWALALGLGLMFEVTHAFRSQGRGSLVVAVGRERKM (SEQ ID NO: 183). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Colon Carcinoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the gastrointestinal tract, such as diseases of the colon, especially colon cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the gastrointestinal tract and/or digestive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, intestinal, colon, cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, feces, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in colon cancer indicates that polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, treatment and/or detection of tumors, especially of the intestine, such as, carcinoid tumors, lymphomas, cancer of the colon and cancer of the rectum, as well as cancers in other tissues where expression has been indicated. Expression in colon carcinoma also indicates the gene or its products would be useful for the diagnosis, treatment and/or prevention of disorders of the colon, including inflammatory disorders such as, diverticular colon disease (DCD), inflammatory colonic disease, Crohn's disease (CD), non-inflammatory bowel disease (non-IBD) colonic inflammation; ulcerative disorders such as, ulcerative colitis (UC), amebic colitis, eosinophilic colitis; noncancerous tumors, such as, polyps in the colon, adenomas, leiomyomas, lipomas, and angiomas. In addition, the expression within cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation. Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain degenerative disorders, such as spinal muscular atrophy (SMA). Alternatively, this gene product may be involved in the pattern of cellular proliferation that accompanies early embryogenesis. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and

would be useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein would be useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 15

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

MILPDPEKPVRLNVKYDKDASFLAGGLFTDFMISVISEDDSIIKNINPAR ISMKMWKLSTSGNRPPANAETFSCNKIKDNDKEDGCFYFRDKVIPNKVGTYC IQFGFMMDKTNILNSEQVIVEVLPNQPVKLVPKIKPPTPAVSNVRSVASRTLV RDLHLSITDDYDNHTGIDLVGTIIATIKGSNEEDTDTPLFIGKVRTLEFPFVNGS AEIMSLVLAESSPGRDSTEYFIVFEPRLPLLSRTLEPYILPFMFYNDVKKQQQM AALTKEKDQLSQSIVMYKSLFEASQQLLNEMKCQVEEARLKEAQLRNELKIH NIDIPTTQQVPHIEALLKRKLSEQEELKKKPRRSCTLPNYTKGSGDVLGKIAHL AQIEDDRAAMVISWHLASDMDCVVTLTTDAARRIYDETQGRQQVLPLDSIYK KTLPDWKRSLPHFRNGKLYFKPIGDPVFARDLLTFPDNVEHCETVFGMLLGD TIILDNLDAANHYRKEVVKITHCPTLLTRDGDRIRSNGKFGGLQNKAPPMDKL RGMVFGAPVPKQCLILGEQIDLLQQYRSAVCKLDSVNKDLNSQLEYLRTPDM RKKKQELDEHEKNLKLIEEKLGMTPIRKCNDSLRHSPKVETTDCPVPPKRMR REATRQNRIITKTDV (SEQ ID NO:184). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent

conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 18. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 18.

This gene is expressed primarily in the following tissues/cDNA libraries: Primary Dendritic Cells, lib 1 and to a lesser extent in Human Bone Marrow, treated; T cell helper II; Lingual Gyrus; Human Soleus; Stratagene placenta (#937225); pBMC stimulated w/ poly I/C; Healing groin wound, 7.5 hours post incision; HL-60, PMA 4H, re-excision; Human Manic Depression Tissue; B-Cells; PC3 Prostate cell line; Primary Dendritic cells,frac 2; Anergic T-cell; T Cell helper I; Hodgkin's Lymphoma II; Human 8 Week Whole Embryo; Keratinocyte; Soares_total_fetus_Nb2HF8_9w and NCI_CGAP_GCB1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the immune system and central nervous system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and/or central nervous systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, neural, neuronal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Elevated levels of this protein are observed in dendrites indicating a role in neurological differentiation. Thus, polynucleotides and polypeptides corresponding to this gene would be useful for the detection, treatment, and/or prevention of

neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival.

In addition, this gene is also highly expressed in various B and T cell populations indicating a role in immune modulation, therefore polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus

erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 16

The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 12.

This gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1NIB and to a lesser extent in normalized infant brain cDNA; Soares_NhHMPu_S1; Human Placenta; Stromal cell TF274; Human Bone Marrow, treated; Soares_total_fetus_Nb2HF8_9w; NCI_CGAP_GCB1; Human Placenta (re-excision); Human Gall Bladder; Human Amygdala; Soares melanocyte 2NbHM; Soares_NFL_T_GBC_S1; Adipose tissue (human); Thyroid Tumour; NCI_CGAP_Br3; Human Adult Retina; HEL cell line; Synovial IL-1/TNF stimulated; Human T-cell lymphoma,re-excision; NCI_CGAP_Lym12; NCI_CGAP_Pr2; Stratagene NT2 neuronal precursor 937230; L428; Human Hypothalmus,Schizophrenia; Human Adipose; Human Ovary; Smooth muscle, serum induced,re-exc; Stratagene colon (#937204); Human T-Cell Lymphoma; Human Testes Tumor; Human Ovarian Cancer Reexcision; Human Placenta; Human Adult Pulmonary,re-excision; Soares_multiple_sclerosis_2NbHMSP; Human 8 Week Whole Embryo; Soares_parathyroid_tumor_NbHPA; Nine Week Old Early Stage

Human; Human Cerebellum; Soares_fetal_liver_spleen_1NFLS_S1; Soares_testis_NHT and Primary Dendritic Cells, lib 1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the central nervous system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous sytem, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., central nervous system, brain, neural, neuronal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal

differentiation or survival. In addition, the polynucleotides and polypeptides corresponding to this gene would be useful for the treatment, detection, and/or prevention of the following non-limiting and exemplary diseases and/or disorders: gall bladder cancer, t-cell lymphoma, ovarian cancer, parathyroid tumor.

FEATURES OF PROTEIN ENCODED BY GENE NO: 17

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: ARGWCLCPFDMTLSVLQHFFICVLLILLLDTNLCRQISSHSFEFSGNQPLVFCCI SSISAKLVLDQAG (SEQ ID NO: 185). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Neutrophils IL-1 and LPS induced.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the immune and/or hematopoietic system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune or hematopoietic systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, hematopoietic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level,

i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in activated neutrophils indicates that polynucleotides and polypeptides corresponding to this clone would be useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes indicates a usefulness in the treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 18

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with several heme receptor proteins (see, e.g., genbank accession no. gb|AAC27029.1| (shown in SEQ ID NO:186) all information available through the recited accession number is hereby incorporated herein by reference). Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group: TLLAMAVAASLSFTVHAETAADASTLDTVRVQAERAKKTRSXNQNV TVLTAADLDNEMANTMEEAIRYIPGVSIVDMGRFGDNGFNIRGLESDRVAITV DGLSLGESVETARSYEFFRGGRGDVDIDTLKSLAVIKGADSISAGSGALGGAV VFTTKDPADYLKPAGNDTHLGFKAGYSGANDETMGTLTFANRTGIVESMLV YTRREGHESESWYDTTNDRIGVGRRTPDPVDSTRDNLLGKLDLQLDEXNTLG FLYERGRATNDVDNLSRVYAPGYLSRKGHDTNDRDRYGVNYQWRADTALF DTLDAQVDRQVTDSRGITTIVAGSGCPGGATPCLXSENRSTKQTLDRAAADF SKVFATAGARHDVVYGLAWQQRDIDFTAVDTRWNAAGAIASVEIDPRQVPK TDVTAWNLYLRDSVQLAGRTXDLSAGARYD (SEQ ID NO: 187), and MKSSLTLLAMAVAASLSFTVHAETAADASTLDTVRVQAERAKKTRSANQNV TVLTAADLDNEMANTMEEAIRYIPGVSIVDMGRFGDNGFNIRGLESDRVAITV DGLSLGESVETARSYEFFRGGRGDVDIDTLKSLAVIKGADSISAGSGALGGAV VFTTKDPADYLKPAGNDTHLGFKAGYSGANDETMGTLTFANRTGIVESMLV YTRREGHESESWYDTTNDRIGVGRRTPDPVDSTRDNLLGKLDLQLDEANTLG FLYERGRATNDVDNLSRVYAPGYLSRKGHDTNDRDRYGVNYQWRADTALF DTLDAQVDRQVTDSRGITTIVAGSGCPGGATPCLRSENRSTKQTLDRAAADFS KVFATAGARHDVVYGLAWQQRDIDFTAVDTRWNAAGAIASVEIDPRQVPKT DVTAWNLYLRDSVQLLDERLTLSAGARYDRYDYSPQVDATFVDRTGTVRDV SFASPSWQAGAEYRFLPDHALWAQVGRGFRAPTVADMYSPTSATQVINAQN

GQPLLLNDTVSNPDLDSEKSLN (SEQ ID NO: 188). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides) are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Human Ovarian Cancer Reexcision.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the female reproductive system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, ovarian, cancerous and wounded tissues) or bodily fluids (e.g., vaginal pool, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in ovarian cancer tissue, indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the treatment and diagnosis of tumors, especially ovarian cancer, as well as cancers of other tissues where expression has been indicated. The expression in ovarian cancer tissue may indicate the gene or its products would be useful in treating, detecting, preventing and/or diagnosing disorders of the ovary, including inflammatory disorders, such as oophoritis (e.g., caused by viral or bacterial infection), ovarian cysts, amenorrhea, infertility, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth). Furthermore, the protein may also be used to determine

biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 19

This gene is expressed primarily in the following tissues/cDNA libraries: Colon Normal II; Soares_multiple_sclerosis_2NbHMSP; NCI_CGAP_GCB1.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the gastrointestinal tract, such as diseases of the colon. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the gastrointestinal tract, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., gastrointestinal, colon, cancerous and wounded tissues) or bodily fluids (e.g., bile, lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in colon indicates the gene or its products would be useful for the diagnosis, treatment and/or prevention of disorders of the colon, including inflammatory disorders such as, diverticular colon disease (DCD), inflammatory colonic disease, Crohn's disease (CD), non-inflammatory bowel disease (non-IBD) colonic inflammation; ulcerative disorders such as, ulcerative colitis (UC), amebic colitis, eosinophilic colitis; noncancerous tumors, such as, polyps in the colon, adenomas, leiomyomas, lipomas, and angiomas. In addition, the tissue distribution in

colon indicates that polynucleotides and polypeptides corresponding to this gene would be useful for diagnosis, treatment and/or detection of tumors, especially of the intestine, such as, carcinoid tumors, lymphomas, cancer of the colon and cancer of the rectum, as well as cancers in other tissues where expression has been indicated. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 20

This gene is expressed primarily in the following tissues/cDNA libraries: Human Ovarian Cancer Reexcision; Soares placenta Nb2HP.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: disorders of the reproductive system and/or vascular disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive and/or vascular, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., reproductive, ovarian, vascular, placental, cancerous and wounded tissues) or bodily fluids (e.g., vaginal pool, amniotic fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in ovarian cancer tissue, indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the treatment and diagnosis of tumors, especially ovarian cancer, as well as cancers of other tissues

where expression has been indicated. The expression in ovarian cancer tissue may indicate the gene or its products can be used to treat and/or diagnose disorders of the ovary, including inflammatory disorders, such as oophoritis (e.g., caused by viral or bacterial infection), ovarian cysts, amenorrhea, infertility, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth). In addition, the expression in placental tissues indicates that polynucleotides and polypeptides corresponding to this clone would be useful in the detection, treatment, and/or prevention of a variety of vascular disorders and conditions, which include, but are not limited to miscrovascular disease, vascular leak syndrome, aneurysm, stroke, embolism, thrombosis, coronary artery disease, arteriosclerosis, and/or atherosclerosis. For example, this gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

FEATURES OF PROTEIN ENCODED BY GENE NO: 21

The translation product of this gene shares sequence identity with heat shock proteins, especially DnaJ proteins from a number of organisms (see, e.g., GenSeq Accession Number Y32858 and Genbank Accession Numbers gb|AAD22362.1|AC006592_19 (AC006592) and gb|AAA68779.1|; all references available through these accession numbers are hereby incorporated by reference herein).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
8	HEEAA16	PTA-847 10/13/99	Uni-ZAP XR	52	1992	1	1984	296	296	132	1	23	24	318
8	HEEAA16	PTA-847 10/13/99	Uni-ZAP XR	53	791	183	791		501	133	1	1	2	97
9	HSPBY63	PTA-847 10/13/99	pSport1	19	2053	1	2053	197	197	99	1	24	25	35
9	HSPBY63	PTA-847 10/13/99	pSport1	54	1265	1	1265	187	187	134	1	24	25	35
9	HSPBY63	PTA-847 10/13/99	pSport1	55	595	1	595		488	135	1	1	2	93
10	HE8QV43	PTA-847 10/13/99	Uni-ZAP XR	20	1672	1	1672	478	478	100	1	20	21	81
10	HE8QV43	PTA-847 10/13/99	Uni-ZAP XR	56	1013	3	1013	472	472	136	1	20	21	81
10	HE8QV43	PTA-847 10/13/99	Uni-ZAP XR	57	701	416	701		396	137	1			9
10	HE8QV43	PTA-847 10/13/99	Uni-ZAP XR	58	814	1	814		34	138	1	12	13	73
11	HWLJX42	PTA-847 10/13/99	pSport1	21	1403	1	1403	211	211	101	1	29	30	42
11	HWLJX42	PTA-847 10/13/99	pSport1	59	1308	489	1308	689	689	139	1	29	30	42

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
11	HWLJX42	PTA-847 10/13/99	pSport1	60	1121	1	1121		440	140	1			7
12	HAPSO15	PTA-847 10/13/99	Uni-ZAP XR	22	1944	1	1941	110	110	102	1	37	38	49
12	HAPSO15	PTA-847 10/13/99	Uni-ZAP XR	61	2013	1	2013	177	177	141	1	37	38	49
12	HAPSO15	PTA-847 10/13/99	Uni-ZAP XR	62	3387	3160	3331		451	142	1	1	2	394
13	HE8QG24	PTA-847 10/13/99	Uni-ZAP XR	23	3059	1	3059	412	412	103	1	23	24	54
13	HE8QG24	PTA-847 10/13/99	Uni-ZAP XR	63	1420	187	1420	508	508	143	1	23	24	85
13	HE8QG24	PTA-847 10/13/99	Uni-ZAP XR	64	1045	1	582		380	144	1	1	2	85
14	HCRNO87	PTA-847 10/13/99	pSport1	24	1769	1	1769	233	233	104	1	25	26	44
14	HCRNO87	PTA-847 10/13/99	pSport1	65	786	1	786	274	274	145	1	25	26	44
14	HCRNO87	PTA-847 10/13/99	pSport1	66	237	1	237		155	146	1			15
14	HCRNO87	PTA-847 10/13/99	pSport1	67	233	1	233		97	147	1	1	2	32

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
15	HBKED12	PTA-847 10/13/99	pSport1	25	3335	1	3335	63	63	105	1	18	19	24
15	HBKED12	PTA-847 10/13/99	pSport1	68	797	1	797	84	84	148	1	18	19	24
15	HBKED12	PTA-847 10/13/99	pSport1	69	1514	1	1514		321	149	1	9	10	20
15	HBKED12	PTA-847 10/13/99	pSport1	70	529	1	529		527	150	1	1	2	175
15	HBKED12	PTA-847 10/13/99	pSport1	71	960	1	680		873	151	1	1	2	156
16	HE8UY36	PTA-847 10/13/99	Uni-ZAP XR	26	2340	1	2340	217	217	106	1	31	32	37
16	HE8UY36	PTA-847 10/13/99	Uni-ZAP XR	72	2393	1	2393	210	210	152	1	31	32	37
17	HNHNT13	PTA-847 10/13/99	Uni-ZAP XR	27	1516	1	1516	32	32	107	1	24	25	58
17	HNHNT13	PTA-847 10/13/99	Uni-ZAP XR	73	748	1	748	25	25	153	1	24	25	58
17	HNHNT13	PTA-847 10/13/99	Uni-ZAP XR	74	991	1	991		661	154	1			2
18	HODEB50	PTA-847 10/13/99	Uni-ZAP XR	28	2880	1	2880	238	238	108	1	21	22	283

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
18	HODEB50	PTA-847 10/13/99	Uni-ZAP XR	75	2751	1	2751	231	231	155	1	21	22	283
18	HODEB50	PTA-847 10/13/99	Uni-ZAP XR	76	774	1	774		403	156	1	11	12	124
19	HWLFQ64	PTA-847 10/13/99	pSport1	29	671	1	671	33	33	109	1	17	18	42
19	HWLFQ64	PTA-847 10/13/99	pSport1	77	655	1	655	84	84	157	1	17	18	42
20	HODFW41	PTA-847 10/13/99	Uni-ZAP XR	30	827	1	827	377	377	110	1			29
20	HODFW41	PTA-847 10/13/99	Uni-ZAP XR	78	748	1	748	370	370	158	1			29
21	HE9RO44	PTA-847 10/13/99	Uni-ZAP XR	31	2322	1	2322	111	111	111	1	25	26	737
21	HE9RO44	PTA-847 10/13/99	Uni-ZAP XR	79	1314	1	1314	185	185	159	1	25	26	332
21	HE9RO44	PTA-847 10/13/99	Uni-ZAP XR	80	612	1	612	612	612	160	1	1	2	204
22	HE9SE18	PTA-847 10/13/99	Uni-ZAP XR	32	2737	1	2737	155	155	112	1	28	29	42
22	HE9SE18	PTA-847 10/13/99	Uni-ZAP XR	81	733	1	733	148	148	161	1	28	29	42

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
22	HE9SE18	PTA-847 10/13/99	Uni-ZAP XR	82	594	1	594		333	162	1	1	2	111
23	HISCV60	PTA-847 10/13/99	pSport1	33	1479	1	1479	19	19	113	1	26	27	128
23	HISCV60	PTA-847 10/13/99	pSport1	83	1484	1	1484	9	9	163	1	26	27	128
24	HNGOI12	PTA-847 10/13/99	Uni-ZAP XR	34	2128	1	2128	27	27	114	1	34	35	57
24	HNGOI12	PTA-847 10/13/99	Uni-ZAP XR	84	774	1	774	27	27	164	1	34	35	57
24	HNGOI12	PTA-847 10/13/99	Uni-ZAP XR	85	1396	1	1396		596	165	1	25	26	93
25	HE8UT25	PTA-847 10/13/99	Uni-ZAP XR	35	2034	1	2034	542	542	115	1	24	25	38
25	HE8UT25	PTA-847 10/13/99	Uni-ZAP XR	86	522	1	522	450	450	166	1			24
25	HE8UT25	PTA-847 10/13/99	Uni-ZAP XR	87	608	1	608		197	167	1	1	2	65
26	HNGMJ91	PTA-847 10/13/99	Uni-ZAP XR	36	638	1	638	224	224	116	1	23	24	57
26	HNGMJ91	PTA-847 10/13/99	Uni-ZAP XR	88	883	1	883		50	168	1			12

Gene No.	cDNA Clone ID	ATCC Deposit No:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
26	HNGMJ91	PTA-847 10/13/99	Uni-ZAP XR	89	765	1	765		764	169	1	1	2	86
27	HNGNB69	PTA-847 10/13/99	Uni-ZAP XR	37	715	1	715	263	263	117	1	30	31	48
28	HNGPM78	PTA-847 10/13/99	Uni-ZAP XR	38	1747	1	1747	307	307	118	1	20	21	25
28	HNGPM78	PTA-847 10/13/99	Uni-ZAP XR	90	1190	1	1190	300	300	170	1	20	21	25

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently

accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed

herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

Table 2 provides preferred epitopes contained in certain embodiments of the invention and polynucleotide sequences that may be disclaimed according to certain embodiments of the invention. The first column refers to each "Gene No." described above in Table 1. The second column provides the sequence identifier, "NT SEQ ID NO:X", for polynucleotide sequences disclosed in Table 1. The third column provides the sequence identifier, "AA SEQ ID NO:Y", for polypeptide sequences disclosed in Table 1. The fourth column provides a unique integer "ntA" where "ntA" is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, and the fifth column provides a unique integer "ntB" where "ntB" is any integer between 15 and the final nucleotide of SEQ ID NO:X, where both ntA and ntB correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where ntB is greater than or equal to a + 14. For each of the polynucleotides shown as SEQ ID NO:X, the uniquely defined integers can be substituted into the general formula of a-b, and used to describe polynucleotides which may be preferably excluded from the invention. Column 6 lists residues comprising predicted epitopes contained in the polypeptides encoded by each of the preferred ORFs (SEQ ID NO:Y). Identification of potential immunogenic regions was performed according to the method of Jameson and Wolf ((1988) CABIOS, 4; 181-186); specifically, the Genetics Computer Group (GCG) implementation of this algorithm, embodied in the program PEPTIDESTRUCTURE

(Wisconsin Package v10.0, Genetics Computer Group (GCG), Madison, Wisc.). This method returns a measure of the probability that a given residue is found on the surface of the protein. Regions where the antigenic index score is greater than 0.9 over at least 6 amino acids are indicated in Table 2 as "Preferred Epitopes". Polypeptides of the invention may possess one, two, three, four, five or more antigenic epitopes comprising residues described in Table 2. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly.

Table 3 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone identifier, "Clone ID", for a cDNA clone related to each contig sequence disclosed in Table 1. Column 2, "Library Codes" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell source identifier code corresponding to the Library Code and Library description provided in Table 4. Expression of these polynucleotides was not observed in the other tissues and/or cell libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

Table 4 provides a key to the Library Code disclosed in Table 3. Column 1 provides the Library Code disclosed in Table 3, column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived. Library codes corresponding to diseased Tissues are indicated in column 3 with the word "disease".

Table 2

Gene #	NT SEQ ID NO: X	AA SEQ ID NO: Y	nt A	nt B	Preferred Epitopes
1	11	91	1 - 2098	15 - 2112	Pro-19 to Gln-32 Lys-45 to Arg-50 Pro-87 to Ser-95 Gly-125 to Glu-130 Cys-140 to Gly-149 Pro-164 to His-173 Glu-229 to Pro-237 Ala-263 to Val-271 Ser-314 to Arg-331 Gly-364 to Ser-370 Arg-388 to Gly-394.
1	39	119	1 - 2229	15 - 2243	Pro-19 to Gln-32 Lys-45 to Arg-50 Pro-87 to Ser-95 Gly-125 to Glu-130 Cys-140 to Gly-149 Pro-164 to His-173 Glu-229 to Pro-237 Ala-263 to Val-271 Ser-314 to Arg-331.
2	12	92	1 - 1825	15 - 1839	
2	40	120	1 - 653	15 - 667	
2	41	121	1 - 774	15 - 788	
2	42	122	1 - 779	15 - 793	Arg-10 to Lys-16.
3	13	93	1 - 2134	15 - 2148	
3	43	123	1 - 991	15 - 1005	
3	44	124	1 - 661	15 - 675	
4	14	94	1 - 2433	15 - 2447	Gln-28 to Gly-36 Thr-96 to Ile-102 Ile-131 to Arg-140 Ser-161 to Asp-170 Pro-357 to Ser-368.
4	45	125	1 - 2072	15 - 2086	Gln-28 to Gly-36 Thr-96 to Ile-102 Ile-131 to Arg-140 Ser-161 to Asp-170.
4	46	126	1 - 708	15 - 722	Pro-11 to Lys-17 Lys-23 to Ser-29 Asn-38 to Ser-43 Asn-67 to Arg-73 Asp-88 to Arg-93.
5	15	95	1 - 3321	15 - 3335	Arg-30 to Ile-42 Asn-103 to Ser-117 Asn-146 to Ser-151 Lys-165 to Asn-171 Asn-177 to Tyr-191 Arg-205 to Met-210 Pro-225 to Ser-236 Pro-254 to His-259

					Asp-391 to Val-396 Lys-402 to Trp-408 Lys-422 to Asp-444 Pro-494 to Glu-502 Gly-517 to Lys-528 Ala-557 to His-566 Pro-568 to Asp-581 Leu-638 to Asn-644 His-651 to Glu-658 His-668 to Ser-681 Phe-685 to Asp-693 Arg-699 to Leu-711 Arg-713 to Cys-720 Thr-729 to Trp-735 Ser-751 to Tyr-757 Asp-783 to Tyr-789 Ser-815 to Asn-828 Gly-836 to Ser-860.
5	47	127	1 - 4289	15 - 4303	Arg-30 to Ile-42 Asn-103 to Ser-117 Asn-146 to Ser-151 Lys-165 to Asn-171 Asn-177 to Tyr-191 Arg-205 to Met-210 Pro-225 to Ser-236 Pro-254 to His-259 Asp-391 to Val-396 Lys-402 to Trp-408 Lys-422 to Asp-444 Pro-494 to Glu-502 Gly-517 to Lys-528.
6	16	96	1 - 1128	15 - 1142	
6	48	128	1 - 1132	15 - 1146	
6	49	129	1 - 144	15 - 158	Glu-1 to Pro-10 Glu-31 to Ala-38.
7	17	97	1 - 1584	15 - 1598	Ser-23 to Trp-30.
7	50	130	1 - 754	15 - 768	Ser-23 to Trp-30.
7	51	131	1 - 1378	15 - 1392	
8	18	98	1 - 1830	15 - 1844	Gly-49 to Lys-63 Ser-88 to Gly-93 Asn-141 to Thr-146 Glu-195 to Phe-201 Val-251 to Ser-259 Gln-267 to Gln-272 Cys-297 to Tyr-302.
8	52	132	1 - 1978	15 - 1992	Gly-49 to Lys-63 Ser-88 to Gly-93 Asn-141 to Thr-146 Glu-195 to Phe-201 Val-251 to Ser-259 Gln-267 to Gln-272 Cys-297 to Asp-304.
8	53	133	1 - 777	15 - 791	
9	19	99	1 - 2039	15 - 2053	
9	54	134	1 - 1251	15 - 1265	
9	55	135	1 - 581	15 - 595	

10	20	100	1 - 1658	15 - 1672	Ser-45 to Gln-53 Lys-61 to Ala-68.
10	56	136	1 - 999	15 - 1013	Ser-45 to Gln-53 Lys-61 to Lys-66.
10	57	137	1 - 687	15 - 701	
10	58	138	1 - 800	15 - 814	Ser-37 to Gln-45 Lys-53 to Ala-60.
11	21	101	1 - 1389	15 - 1403	Phe-28 to Arg-34.
11	59	139	1 - 1294	15 - 1308	Phe-28 to Arg-34.
11	60	140	1 - 1107	15 - 1121	
12	22	102	1 - 1930	15 - 1944	
12	61	141	1 - 1999	15 - 2013	
12	62	142	1 - 3373	15 - 3387	Leu-18 to Asp-26 Thr-43 to Met-53 Glu-55 to Asp-66 Ser-68 to Lys-86 Ala-113 to Arg-118 Gln-121 to Asp-130 Ala-209 to Lys-216 Gly-270 to Lys-275 Asn-309 to Lys-314 Lys-332 to Gln-342.
13	23	103	1 - 3045	15 - 3059	
13	63	143	1 - 1406	15 - 1420	
13	64	144	1 - 1031	15 - 1045	Ala-73 to Ile-81.
14	24	104	1 - 1755	15 - 1769	
14	65	145	1 - 772	15 - 786	
14	66	146	1 - 223	15 - 237	Pro-10 to Gln-15.
14	67	147	1 - 219	15 - 233	Lys-16 to Pro-32.
15	25	105	1 - 3321	15 - 3335	
15	68	148	1 - 783	15 - 797	
15	69	149	1 - 1500	15 - 1514	Thr-7 to Gln-13.
15	70	150	1 - 515	15 - 529	Arg-37 to Phe-48 Gly-50 to Pro-57.
15	71	151	1 - 946	15 - 960	Arg-13 to Phe-24 Gly-26 to Pro-33 Ser-72 to Leu-77 Arg-85 to Lys-101.
16	26	106	1 - 2326	15 - 2340	
16	72	152	1 - 2379	15 - 2393	
17	27	107	1 - 1502	15 - 1516	
17	73	153	1 - 734	15 - 748	
17	74	154	1 - 977	15 - 991	
18	28	108	1 - 2866	15 - 2880	Arg-58 to Val-79 Leu-98 to Arg-104 Leu-108 to Asn-113 Glu-133 to Gln-140 Ser-146 to Lys-152 Asp-191 to Gly-196 Arg-210 to Val-218 Asp-234 to Gly-239 Asp-255 to Asp-262.
18	75	155	1 - 2737	15 - 2751	Arg-58 to Val-79 Leu-98 to Arg-104 Leu-108 to Asn-113

					Glu-133 to Gln-140 Ser-146 to Lys-152 Asp-167 to Gly-172.
18	76	156	1 - 760	15 - 774	
19	29	109	1 - 657	15 - 671	
19	77	157	1 - 641	15 - 655	
20	30	110	1 - 813	15 - 827	
20	78	158	1 - 734	15 - 748	
21	31	111	1 - 2308	15 - 2322	Ala-43 to Leu-52 His-57 to Glu-67 Ser-81 to Arg-113 Asn-131 to Glu-140 Trp-266 to Lys-271 Gly-309 to Ile-319 Pro-376 to Lys-385 Glu-394 to Lys-400 Ser-424 to Phe-430 Glu-451 to Ala-456 Asp-483 to Pro-489 Ser-560 to Lys-597 Pro-682 to Lys-687 Lys-712 to Glu-719.
21	79	159	1 - 1300	15 - 1314	Ala-43 to Leu-52 His-57 to Glu-67 Ser-81 to Arg-113 Asn-131 to Glu-140.
21	80	160	1 - 598	15 - 612	Ser-25 to Lys-62 Pro-147 to Lys-152 Lys-177 to Glu-184.
22	32	112	1 - 2723	15 - 2737	
22	81	161	1 - 719	15 - 733	
22	82	162	1 - 580	15 - 594	Gly-2 to Trp-8 Val-49 to Gly-110.
23	33	113	1 - 1465	15 - 1479	Gln-107 to Phe-112.
23	83	163	1 - 1470	15 - 1484	
24	34	114	1 - 2114	15 - 2128	Met-1 to Gly-9.
24	84	164	1 - 760	15 - 774	Met-1 to Gly-9.
24	85	165	1 - 1382	15 - 1396	
25	35	115	1 - 2020	15 - 2034	
25	86	166	1 - 508	15 - 522	
25	87	167	1 - 594	15 - 608	Lys-60 to Asn-65.
26	36	116	1 - 624	15 - 638	Gly-30 to Asp-38.
26	88	168	1 - 869	15 - 883	
26	89	169	1 - 751	15 - 765	
27	37	117	1 - 701	15 - 715	
28	38	118	1 - 1733	15 - 1747	Thr-11 to Ser-17.
28	90	170	1 - 1176	15 - 1190	

Table 3

Clone ID	Library Codes
HWLFJ10	H0031 H0265 H0436 H0542 H0634 L1290 S0038 S0114 S0126 S0354 S0454
HEEAM62	H0549
HWLEC41	H0008 H0012 H0038 H0041 H0050 H0052 H0057 H0068 H0071 H0081 H0083 H0090 H0123 H0125 H0134 H0136 H0156 H0165 H0167 H0178 H0181 H0194 H0253 H0255 H0265 H0351 H0352 H0355 H0412 H0422 H0423 H0428 H0510 H0529 H0530 H0540 H0542 H0543 H0545 H0550 H0555 H0556 H0559 H0581 H0584 H0616 H0618 H0619 H0620 H0644 H0656 H0657 H0682 H0685 H0686 H0696 L1290 S0206 S0328 S0330 S0354 S0356 S0376 S0420 S0424 S0468 S3014 T0006 T0039 T0042
HWLFR02	H0264 H0519 H0551 H0595 H0619 H0665 H0670 S0126 S0338 S0340 S0354 S0390
HE9QN39	H0013 H0039 H0046 H0050 H0052 H0144 H0244 H0251 H0309 H0316 H0318 H0328 H0331 H0351 H0352 H0370 H0372 H0380 H0411 H0428 H0486 H0494 H0518 H0521 H0522 H0547 H0553 H0580 H0581 H0586 H0587 H0593 H0622 H0633 H0638 H0658 H0670 H0691 H0704 L1290 S0042 S0045 S0046 S0051 S0144 S0216 S0294 S0330 S0344 S0354 S0360 S0376 S0378 S6028 T0049 T0104
HNHKL90	S0216
HSXEQ06	H0013 H0036 H0038 H0050 H0090 H0098 H0170 H0171 H0341 H0423 H0427 H0436 H0444 H0457 H0486 H0539 H0543 H0586 H0587 H0590 H0623 H0624 H0625 H0632 H0633 H0635 H0657 H0658 H0659 L1290 S0001 S0003 S0010 S0027 S0036 S0045 S0222 S0242 S0330 S0418 S0426 S0434 T0003
HEEAA16	H0013 H0024 H0031 H0032 H0038 H0050 H0051 H0098 H0135 H0136 H0144 H0163 H0170 H0208 H0327 H0328 H0341 H0351 H0369 H0422 H0435 H0436 H0444 H0488 H0494 H0497 H0509 H0519 H0520 H0539 H0543 H0545 H0547 H0549 H0553 H0555 H0556 H0563 H0574 H0575 H0580 H0581 H0586 H0587 H0591 H0592 H0616 H0620 H0623 H0624 H0627 H0628 H0634 H0635 H0637 H0648 H0659 H0660 H0668 H0670 H0674 H0685 H0686 H0690 L1290 S0002 S0003 S0011 S0026 S0027 S0028 S0031 S0032 S0036 S0045 S0046 S0049 S0051 S0052 S0132 S0142 S0194 S0206 S0214 S0222 S0276 S0326 S0328 S0330 S0344 S0354 S0356 S0358 S0366 S0372 S0374 S0376 S0380 S0406 S0414 S0420 S0424 S0426 S0434 T0006
HSPBY63	H0478
HE8QV43	H0013 H0144 H0169 H0208 H0268 H0428 H0539 H0543 H0623 L1290 S0152 S0300
HWLJX42	H0331 H0357 H0574 H0616 H0687 L1290 S0276 S0280 S0328 S0356 S0358
HAPSO15	H0004 H0013 H0014 H0031 H0032 H0046 H0056 H0144 H0170 H0171 H0251 H0264 H0309 H0341 H0411 H0423 H0427 H0486 H0497 H0521 H0546 H0551 H0555 H0574 H0575 H0586 H0598 H0615 H0622 H0624 H0632 H0644 H0653 H0656 H0657 H0661 H0666 H0670 L1290 S0001 S0003 S0010 S0011 S0013 S0039 S0114 S0126 S0134 S0144 S0152 S0242 S0282 S0356 S0374 S0376 S0378 S0450 S3012 S6026 S6028
HE8QG24	H0013 H0250 H0305 H0522 H0550 H0589 H0619 L1290 S0010 S0036 S0196 S0222 S0418 T0068
HCRNO87	H0100 H0144 H0170 H0254 H0264 H0305 H0341 H0352 H0402 H0406 H0414 H0422 H0428 H0486 H0519 H0545 H0580 H0581 H0617 H0619 H0672 H0692 L1290 S0010 S0051 S0356 S0358 S0360 S0392 T0115

HBKED12	H0013 H0052 H0070 H0274 H0342 H0402 H0421 H0422 H0423 H0436 H0457 H0486 H0494 H0518 H0521 H0522 H0539 H0542 H0543 H0547 H0550 H0551 H0555 H0559 H0580 H0581 H0591 H0610 H0615 H0641 H0646 H0650 H0672 H0688 H0696 L1290 S0150 S0152 S0212 S0294 S0328 S0330 S0366 S0382 S0442 S6028
HE8UY36	H0013 H0014 H0031 H0040 H0052 H0090 H0144 H0344 H0427 H0428 H0497 H0521 H0545 H0553 H0561 H0575 H0581 H0587 H0591 H0615 H0644 H0658 H0687 L1290 S0010 S0026 S0051 S0196 S0460 S3014 T0082
HNHNT13	S0216
HODEB50	H0615
HWLFQ64	L1290 S0354
HODFW41	H0615 L1290
HE9RO44	H0013 H0144 H0551 L1290 T0041
HE9SE18	H0144 H0506
HISCV60	H0539
HNGOI12	S0428
HE8UT25	H0013 L1290
HNGMJ91	S0428
HNGNB69	S0428
HNGPM78	S0001 S0428

Table 4

Library Code	Library Description	Disease
H0004	Human Adult Spleen	
H0008	Whole 6 Week Old Embryo	
H0012	Human Fetal Kidney	
H0013	Human 8 Week Whole Embryo	
H0014	Human Gall Bladder	
H0024	Human Fetal Lung III	
H0031	Human Placenta	
H0032	Human Prostate	
H0036	Human Adult Small Intestine	
H0038	Human Testes	
H0039	Human Pancreas Tumor	disease
H0040	Human Testes Tumor	disease
H0041	Human Fetal Bone	
H0046	Human Endometrial Tumor	disease
H0050	Human Fetal Heart	
H0051	Human Hippocampus	
H0052	Human Cerebellum	
H0056	Human Umbilical Vein, Endo. remake	
H0057	Human Fetal Spleen	
H0068	Human Skin Tumor	disease
H0070	Human Pancreas	
H0071	Human Infant Adrenal Gland	
H0081	Human Fetal Epithelium (Skin)	
H0083	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	
H0090	Human T-Cell Lymphoma	disease
H0098	Human Adult Liver, subtracted	
H0100	Human Whole Six Week Old Embryo	
H0123	Human Fetal Dura Mater	
H0125	Cem cells cyclohexamide treated	
H0134	Raji Cells, cyclohexamide treated	
H0135	Human Synovial Sarcoma	
H0136	Supt Cells, cyclohexamide treated	
H0144	Nine Week Old Early Stage Human	
H0156	Human Adrenal Gland Tumor	disease
H0163	Human Synovium	
H0165	Human Prostate Cancer, Stage B2	disease
H0167	Activated T-Cells, 24 hrs.	
H0169	Human Prostate Cancer, Stage C fraction	disease
H0170	12 Week Old Early Stage Human	
H0171	12 Week Old Early Stage Human, II	
H0178	Human Fetal Brain	
H0181	Human Primary Breast Cancer	disease
H0194	Human Cerebellum, subtracted	
H0208	Early Stage Human Lung, subtracted	
H0244	Human 8 Week Whole Embryo, subtracted	
H0250	Human Activated Monocytes	
H0251	Human Chondrosarcoma	disease
H0253	Human adult testis, large inserts	
H0254	Breast Lymph node cDNA library	

H0255	breast lymph node CDNA library	
H0264	human tonsils	
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco	
H0268	Human Umbilical Vein Endothelial Cells, fract. A	
H0274	Human Adult Spleen, fractionII	
H0305	CD34 positive cells (Cord Blood)	
H0309	Human Chronic Synovitis	disease
H0316	HUMAN STOMACH	
H0318	HUMAN B CELL LYMPHOMA	disease
H0327	human corpus colosum	
H0328	human ovarian cancer	disease
H0331	Hepatocellular Tumor	disease
H0341	Bone Marrow Cell Line (RS4,11)	
H0342	Lingual Gyrus	
H0344	Adipose tissue (human)	
H0351	Glioblastoma	disease
H0352	wilm's tumor	disease
H0355	Human Liver	
H0357	H. Normalized Fetal Liver, II	
H0369	H. Atrophic Endometrium	
H0370	H. Lymph node breast Cancer	disease
H0372	Human Testes	
H0380	Human Tongue, frac 2	
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision	
H0406	H Amygdala Depression, subtracted	
H0411	H Female Bladder, Adult	
H0412	Human umbilical vein endothelial cells, IL-4 induced	
H0414	Ovarian Tumor I, OV5232	disease
H0421	Human Bone Marrow, re-excision	
H0422	T-Cell PHA 16 hrs	
H0423	T-Cell PHA 24 hrs	
H0427	Human Adipose	
H0428	Human Ovary	
H0435	Ovarian Tumor 10-3-95	
H0436	Resting T-Cell Library,II	
H0444	Spleen metastic melanoma	disease
H0457	Human Eosinophils	
H0478	Salivary Gland, Lib 2	
H0486	Hodgkin's Lymphoma II	disease
H0488	Human Tonsils, Lib 2	
H0494	Keratinocyte	
H0497	HEL cell line	
H0506	Ulcerative Colitis	
H0509	Liver, Hepatoma	disease
H0510	Human Liver, normal	
H0518	pBMC stimulated w/ poly I/C	
H0519	NTERA2, control	
H0520	NTERA2 + retinoic acid, 14 days	
H0521	Primary Dendritic Cells, lib 1	
H0522	Primary Dendritic cells,frac 2	
H0529	Myoloid Progenitor Cell Line	
H0530	Human Dermal Endothelial Cells,untreated	
H0539	Pancreas Islet Cell Tumor	disease

H0540	Skin, burned	
H0542	T Cell helper I	
H0543	T cell helper II	
H0545	Human endometrial stromal cells-treated with progesterone	
H0546	Human endometrial stromal cells-treated with estradiol	
H0547	NTERA2 teratocarcinoma cell line+retinoic acid (14 days)	
H0549	H. Epididiymus, caput & corpus	
H0550	H. Epididiymus, cauda	
H0551	Human Thymus Stromal Cells	
H0553	Human Placenta	
H0555	Rejected Kidney, lib 4	disease
H0556	Activated T-cell(12h)/Thiouridine-re-excision	
H0559	HL-60, PMA 4H, re-excision	
H0561	L428	
H0563	Human Fetal Brain, normalized 50021F	
H0574	Hepatocellular Tumor, re-excision	disease
H0575	Human Adult Pulmonary,re-excision	
H0580	Dendritic cells, pooled	
H0581	Human Bone Marrow, treated	
H0584	Activated T-cells, 24 hrs,re-excision	
H0586	Healing groin wound, 6.5 hours post incision	disease
H0587	Healing groin wound, 7.5 hours post incision	disease
H0589	CD34 positive cells (cord blood),re-ex	
H0590	Human adult small intestine,re-excision	
H0591	Human T-cell lymphoma,re-excision	disease
H0592	Healing groin wound - zero hr post-incision (control).	disease
H0593	Olfactory epithelium,nasalcavity	
H0595	Stomach cancer (human),re-excision	disease
H0598	Human Stomach,re-excision	
H0610	H. Leukocytes, normalized cot 5A	
H0615	Human Ovarian Cancer Reexcision	disease
H0616	Human Testes, Reexcision	
H0617	Human Primary Breast Cancer Reexcision	disease
H0618	Human Adult Testes, Large Inserts, Reexcision	
H0619	Fetal Heart	
H0620	Human Fetal Kidney, Reexcision	
H0622	Human Pancreas Tumor, Reexcision	disease
H0623	Human Umbilical Vein, Reexcision	
H0624	12 Week Early Stage Human II, Reexcision	
H0625	Ku 812F Basophils Line	
H0627	Saos2 Cells, Vitamin D3 Treated	
H0628	Human Pre-Differentiated Adipocytes	
H0632	Hepatocellular Tumor,re-excision	
H0633	Lung Carcinoma A549 TNFalpha activated	disease
H0634	Human Testes Tumor, re-excision	disease
H0635	Human Activated T-Cells, re-excision	
H0637	Dendritic Cells From CD34 Cells	
H0638	CD40 activated monocyte dendridic cells	
H0641	LPS activated derived dendritic cells	
H0644	Human Placenta (re-excision)	
H0646	Lung, Cancer (4005313 A3): Invasive Poorly Differentiated Lung Adenocarcinoma,	
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot	disease

H0650	B-Cells	
H0653	Stromal Cells	
H0656	B-cells (unstimulated)	
H0657	B-cells (stimulated)	
H0658	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	disease
H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	disease
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	disease
H0661	Breast, Cancer: (4004943 A5)	disease
H0665	Stromal cells 3.88	
H0666	Ovary, Cancer: (4004332 A2)	disease
H0668	stromal cell clone 2.5	
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	
H0672	Ovary, Cancer: (4004576 A8)	
H0674	Human Prostate Cancer, Stage C, re-excission	
H0682	Ovarian cancer, Serous Papillary Adenocarcinoma	
H0685	Adenocarcinoma of Ovary, Human Cell Line, # OVCAR-3	
H0686	Adenocarcinoma of Ovary, Human Cell Line	
H0687	Human normal ovary(#9610G215)	
H0688	Human Ovarian Cancer(#9807G017)	
H0690	Ovarian Cancer, # 9702G001	
H0691	Normal Ovary, #9710G208	
H0692	BLyS Receptor from Expression Cloning	
H0696	Prostate Adenocarcinoma	
H0704	Prostate Adenocarcinoma cell line cultured in vivo in mice	
L1290	NCI_CGAP_Brn25	
S0001	Brain frontal cortex	
S0002	Monocyte activated	
S0003	Human Osteoclastoma	disease
S0010	Human Amygdala	
S0011	STROMAL -OSTEOCLASTOMA	disease
S0013	Prostate	
S0026	Stromal cell TF274	
S0027	Smooth muscle, serum treated	
S0028	Smooth muscle,control	
S0031	Spinal cord	
S0032	Smooth muscle-ILb induced	
S0036	Human Substantia Nigra	
S0038	Human Whole Brain #2 - Oligo dT > 1.5Kb	
S0039	Hypothalamus	
S0042	Testes	
S0045	Endothelial cells-control	
S0046	Endothelial-induced	
S0049	Human Brain, Striatum	
S0051	Human Hypothalmus,Schizophrenia	disease
S0052	neutrophils control	
S0114	Anergic T-cell	
S0126	Osteoblasts	
S0132	Epithelial-TNFa and INF induced	
S0134	Apoptotic T-cell	
S0142	Macrophage-oxLDL	
S0144	Macrophage (GM-CSF treated)	
S0150	LNCAP prostate cell line	

S0152	PC3 Prostate cell line	
S0194	Synovial hypoxia	
S0196	Synovial IL-1/TNF stimulated	
S0206	Smooth Muscle- HASTE normalized	
S0212	Bone Marrow Stromal Cell, untreated	
S0214	Human Osteoclastoma, re-excision	disease
S0216	Neutrophils IL-1 and LPS induced	
S0222	H. Frontal cortex,epileptic,re-excision	disease
S0242	Synovial Fibroblasts (Il1/TNF), subt	
S0276	Synovial hypoxia-RSF subtracted	
S0280	Human Adipose Tissue, re-excision	
S0282	Brain Frontal Cortex, re-excision	
S0294	Larynx tumor	disease
S0300	Frontal lobe,dementia,re-excision	
S0326	Mammary Gland	
S0328	Palate carcinoma	disease
S0330	Palate normal	
S0338	Human Osteoarthritic Cartilage Fraction III	disease
S0340	Human Osteoarthritic Cartilage Fraction IV	disease
S0344	Macrophage-oxLDL, re-excision	
S0354	Colon Normal II	
S0356	Colon Carcinoma	disease
S0358	Colon Normal III	
S0360	Colon Tumor II	disease
S0366	Human Soleus	
S0372	Larynx carcinoma III	disease
S0374	Normal colon	
S0376	Colon Tumor	disease
S0378	Pancreas normal PCA4 No	
S0380	Pancreas Tumor PCA4 Tu	disease
S0382	Larynx carcinoma IV	disease
S0390	Smooth muscle, control, re-excision	
S0392	Salivary Gland	
S0406	Rectum tumour	
S0414	Hippocampus, Alzheimer Subtracted	
S0418	CHME Cell Line,treated 5 hrs	
S0420	CHME Cell Line,untreated	
S0424	TF-1 Cell Line GM-CSF Treated	
S0426	Monocyte activated, re-excision	
S0428	Neutrophils control, re-excision	
S0434	Stomach Normal	disease
S0442	Colon Normal	
S0450	Larynx Tumour	
S0454	Placenta	
S0460	Thyroid Tumour	
S0468	Ea.hy.926 cell line	
S3012	Smooth Muscle Serum Treated, Norm	
S3014	Smooth muscle, serum induced,re-exc	
S6026	Frontal Lobe, Dementia	
S6028	Human Manic Depression Tissue	disease
T0003	Human Fetal Lung	
T0006	Human Pineal Gland	
T0039	HSA 172 Cells	

T0041	Jurkat T-cell G1 phase	
T0042	Jurkat T-Cell, S phase	
T0049	Aorta endothelial cells + TNF-a	
T0068	Normal Ovary, Premenopausal	
T0082	Human Adult Retina	
T0104	HCC cell line metastisis to liver	
T0115	Human Colon Carcinoma (HCC) cell line	

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification , such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the

polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretary leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence

shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as desribed below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the

complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown inTable 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the presence invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp.

App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identiy are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the lenght of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the

deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determing the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window

Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not

matched/aligned with the query sequnce are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over

3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including, but not limited to, recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt,

and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40,

41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel

chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies

described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to

an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant

albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721;

5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin

molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be

excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6}M, 5×10^{-7} M, 10^{7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, $^{10-12}$ M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the

epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferrably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.

5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of

numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an

immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage

gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol.

Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into

mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that “mimic” polypeptides of the invention using

techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library

generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino

acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a

polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as

bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such

fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt,

which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any

combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to

identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin,

and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol., 6*:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific

epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al., Cell, 96*:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A

and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes

the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any

one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention,

including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other

desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination

(Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages,

neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment;

this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by

use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of

the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,

whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods

including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with

a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may

also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound

recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)

Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of nucleic acids which encode these fusion proteins are also encompassed by the invention.

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A,

pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ,pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-

mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O_2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O_2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. *See*, Ellis, S.B., *et al., Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J, *et al., Yeast* 5:167-77 (1989); Tschopp, J.F., *et al., Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a

polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for

derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al., Appl. Biochem. Biotechnol. 56*:59-72 (1996); Vorobjev *et al., Nucleosides Nucleotides 18*:2745-2750 (1999); and Caliceti *et al., Bioconjug. Chem. 10*:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may

be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992); Francis *et al., Intern. J. of Hematol. 68*:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($ClSO_2CH_2CF_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,

15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional

embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627

(hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide seuqence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers

of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hyrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into

liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety)..

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see

Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or

estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferrably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine,

thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal

cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression,CRCPress, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456

(1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant,urine,fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression

in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor supressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also

be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treatingor preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver,

and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA

sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked*nucleic* acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc.

Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology , 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a

suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+}-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell , 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA , 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA , 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis

virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartzet al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al.,Science , 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993);

Rosenfeld et al., Cell , 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature , 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including

lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5′ end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5′ and 3′ ends. Preferably, the 3′ end of the first targeting sequence contains the same restriction enzyme site as the 5′ end of the amplified promoter and the 5′ end of the second targeting sequence contains the same restriction site as the 3′ end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiongenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5′ end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., “gene guns”), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid

(tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

Biological Activities

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Immune Activity

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of

the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g., agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing autoimmune disorders. Many autoimmune disorders result from inappropriate

recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, and/or diagnosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following: autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g, IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erhythematosus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, schleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes millitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiotomy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulamatous, degenerative, and atrophic disorders.

Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are

not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes millitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitchondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiotomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulamatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated,

prevented, and/or diagnosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

B cell immunodeficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVI) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic alymophoplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

T cell deficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof include, but are not limited to, for example, DiGeorge anomaly, thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia,

immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity. In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are ameliorated or treated by, for example, administering the polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be ameliorated or treated by administering polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID; e.g., X-linked SCID, autosomal SCID, and adenosine deaminase deficiency), ataxia-telangiectasia, Wiskott-Aldrich syndrome, short-limber dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome (e.g., purine nucleoside phosphorylase deficiency), MHC Class II deficiency. In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof.

In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, systemic lupus erythemosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using antibodies against the protein of the invention.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed using polypeptides, antibodies, or polynucleotides of the invention, and/or

agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Moreover, inflammatory conditions may also be treated, diagnosed, and/or prevented with polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. Such inflammatory conditions include, but are not limited to, for example, respiratory disorders (such as, e.g., asthma and allergy); gastrointestinal disorders (such as, e.g., inflammatory bowel disease); cancers (such as, e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (such as, e.g., multiple sclerosis, blood-brain barrier permeability, ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders (such as, e.g., Parkinson's disease and Alzheimer's disease), AIDS-related dementia, and prion disease); cardiovascular disorders (such as, e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (such as, e.g., chronic hepatitis (B and C), rheumatoid arthritis, gout, trauma, septic shock, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosis, diabetes mellitus (i.e., type 1 diabetes), and allogenic transplant rejection).

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to treat, diagnose, and/or prevent transplantation rejections, graft-versus-host disease, autoimmune and inflammatory diseases (e.g., immune complex-induced vasculitis, glomerulonephritis, hemolytic anemia, myasthenia gravis, type II collagen-induced arthritis, experimental allergic and hyperacute xenograft rejection, rheumatoid arthritis, and systemic lupus erythematosus (SLE). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also be used to modulate and/or diagnose inflammation. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to treat, diagnose, or prognose, inflammatory conditions, both chronic and acute conditions, including, but not limited to, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, and resulting from over production of cytokines (e.g., TNF or IL-1.).

Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

Additional preferred embodiments of the invention include, but are not limited to, the use of polypeptides, antibodies, polynucleotides and/or agonists or antagonists in the following applications:

Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.

Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741.

A vaccine adjuvant that enhances immune responsiveness to specific antigen.

An adjuvant to enhance tumor-specific immune responses.

An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever.

An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae, Mycobacterium leprae, Salmonella typhi, Salmonella paratyphi, Meisseria meningitidis,*

Streptococcus pneumoniae, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli,* Enterohemorrhagic *E. coli*, *Borrelia burgdorferi*, and Plasmodium (malaria).

An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria).

As a stimulator of B cell responsiveness to pathogens.

As an activator of T cells.

As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

As an agent to induce higher affinity antibodies.

As an agent to increase serum immunoglobulin concentrations.

As an agent to accelerate recovery of immunocompromised individuals.

As an agent to boost immunoresponsiveness among aged populations.

As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are

not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery.

As a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, said enhancement or antagonization of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

As an agent to direct an individuals immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodificiency.

As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

As an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

As a means of activating T cells.

As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

As a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists thereof, may be used to treat or prevent IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

All of the above described applications as they may apply to veterinary medicine.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, or ribozymes. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens.

A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and MS.

An inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune responses, and blocking sepsis.

An inhibitor of graft versus host disease or transplant rejection.

A therapy for B cell and/or T cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

A therapy for chronic hypergammaglobulinemeia evident in such diseases as monoclonalgammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonalgammopathies, and plasmacytomas.

A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

An immunosuppressive agent(s).

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

In another embodiment, administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention, may be used to treat or prevent IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

The agonists or antagonists may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes. The antagonists or agonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by, for example, preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration. The antagonists or agonists or may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

Antibodies against polypeptides of the invention may be employed to treat ARDS.

Agonists and/or antagonists of the invention also have uses in stimulating wound and tissue repair, stimulating angiogenesis, stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to treat or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carnii.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to treat, diagnose, and/or prevent (1) cancers or neoplasms and (2) autoimmune cell or tissue-related cancers or neoplasms. In a preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent acute myelogeneous leukemia. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent, chronic myelogeneous leukemia, multiple myeloma, non-Hodgkins lymphoma, and/or Hodgkins disease.

In another specific embodiment, polynucleotides or polypeptides, and/or agonists or antagonists of the invention may be used to treat, diagnose, prognose, and/or prevent selective IgA deficiency, myeloperoxidase deficiency, C2 deficiency, ataxia-telangiectasia, DiGeorge anomaly, common variable immunodeficiency (CVI),

X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome.

Examples of autoimmune disorders that can be treated or detected are described above and also include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prognosed, prevented, and/or diagnosed using antibodies against the polypeptide of the invention.

As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

Additionally, polynucleotides, polypeptides, and/or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related

glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastisis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and

brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be detected and/or treated by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to neoplasms located in the: liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Hyperproliferative Disorders

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals

comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the poynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferrably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature

320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragements thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragements thereof. Preferred binding affinities include those

with a dissociation constant or Kd less than $5X10^{-6}M$, $10^{-6}M$, $5X10^{-7}M$, $10^{-7}M$, $5X10^{-8}M$, $10^{-8}M$, $5X10^{-9}M$, $10^{-9}M$, $5X10^{-10}M$, $10^{-10}M$, $5X10^{-11}M$, $10^{-11}M$, $5X10^{-12}M$, $10^{-12}M$, $5X10^{-13}M$, $10^{-13}M$, $5X10^{-14}M$, $10^{-14}M$, $5X10^{-15}M$, and $10^{-15}M$.

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuviants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such thereapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodes associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodes of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus

arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural

hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromoboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* *56*:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound

healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses *et al., Biotech. 9*:630-634 (1991); Folkman *et al., N. Engl. J. Med., 333*:1757-1763 (1995); Auerbach *et al., J. Microvasc. Res. 29*:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Opthalmol. 94*:715-743 (1982); and Folkman *et al.,* Science *221*:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science 235*:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.,* Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treator prevent a cancer or tumor. Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors,

including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising

the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al., Am. J. Ophthal. 85*:704-710 (1978) and Gartner *et al., Surv. Ophthal. 22*:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of

diseases, disorders, and/or conditions can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-

3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreous injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome,

pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized

to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive

Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence

of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or

agonists or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren’s syndrome, Hashimoto’s thyroiditis, biliary cirrhosis,

Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associted with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft,

epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intesting, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or

agonists or antagonists of the invention, could also be used to treat gastric and doudenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflamamatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and,

thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is

destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists

or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-

Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Further, polypeptides or polynucleotides of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including polynucleotides, polypeptides, and agonists or antagonists) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles, including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such neurodegenerative disease states and/or behavioral disorders include, but are not limited to, Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Additionally, polypeptides, polynucleotides and/or agonists or antagonists of the invention, may be useful in protecting neural cells from diseases, damage, disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to

stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-Spatz Syndrome.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, and cerebral malaria.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include meningitis such as arachnoiditis, aseptic meningtitis such as viral meningtitis which includes lymphocytic choriomeningitis, Bacterial meningtitis which includes Haemophilus Meningtitis, Listeria Meningtitis, Meningococcal Meningtitis such as

Waterhouse-Friderichsen Syndrome, Pneumococcal Meningtitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningtitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie), and cerebral toxoplasmosis.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral sceloris which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon-Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity,

encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron

disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyloclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia,Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.

Additional neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

Infectious Disease

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented, and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or

diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi), Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea,

meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diptheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used totreat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-

Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

Chemotaxis

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used totreat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein

polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, S. Trends Biotechnol. 16(2):76-82 (1998); Hansson, L. O., et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide

sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be alterred by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of

3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity , and (b) determining if a biological activity of the polypeptide has been altered.

Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of,

any combination or all of contained in the polypeptide sequences of the invention. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes

known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the

present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Polypeptides of the Invention Binding Peptides and Other Molecules

The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind polypeptides of the invention, and the polypeptide of the invention binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the polypeptides of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of:

a. contacting a polypeptide of the invention with a plurality of molecules; and
b. identifying a molecule that binds the polypeptide of the invention.

The step of contacting the polypeptide of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the polypeptide of the invention on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptide of the invention. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized polypeptide of the invention. The molecules having a selective affinity for the polypeptide of the invention can then be purified by affinity selection. The nature of the solid support, process for attachment of the polypeptide of the invention to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the polypeptide of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the polypeptide of the invention and the individual clone. Prior to contacting the polypeptide of the invention with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for a polypeptide of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for the polypeptide of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be

determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound polypeptide of the invention, or altemtatively, unbound polypeptides, from a mixture of the polypeptide of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the polypeptide of the invention or the plurality of polypeptides is bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind to a polypeptide of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and in vitro translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., 1991, Science 251:767-773; Houghten et al., 1991, Nature 354:84-86; Lam et al., 1991, Nature 354:82-84; Medynski, 1994, Bio/Technology 12:709-710;Gallop et al., 1994, J. Medicinal Chemistry 37(9):1233-1251; Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422-11426; Houghten et al., 1992, Biotechniques 13:412; Jayawickreme et al., 1994, Proc. Natl. Acad. Sci. USA 91:1614-1618; Salmon et al., 1993, Proc. Natl. Acad. Sci. USA 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383.

Examples of phage display libraries are described in Scott and Smith, 1990, Science 249:386-390; Devlin et al., 1990, Science, 249:404-406; Christian, R. B., et al., 1992, J. Mol. Biol. 227:711-718); Lenstra, 1992, J. Immunol. Meth. 152:149-157; Kay et al., 1993, Gene 128:59-65; and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, Bio/Technology 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates, pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390; Fowlkes et al., 1992; BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al.,

1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al., 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, Science 263:671-673; and CT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds a polypeptide of the invention can be carried out by contacting the library members with a polypeptide of the invention immobilized on a solid phase and harvesting those library members that bind to the polypeptide of the invention. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, Gene 73:305-318; Fowlkes et al., 1992, BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, Nature 340:245-246; Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88:9578-9582) can be used to identify molecules that specifically bind to a polypeptide of the invention.

Where the polypeptide of the invention binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries, such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

As mentioned above, in the case of a polypeptide of the invention binding

molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a polypeptide of the invention binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

The selected polypeptide of the invention binding polypeptide can be obtained by chemical synthesis or recombinant expression.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Anitsense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5 end and a HindIII site on the 3 end. Next, the pair of oligonucleotides is

heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl2, 10MM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature, 29:304-310 (1981), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., Nature, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA,

forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5′ end of the message, *e.g.*, the 5′ untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3′ untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5′ - or 3′ - non- translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5′ untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5′ -, 3′ - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556 (1989); Lemaitre et al., Proc. Natl. Acad. Sci., 84:648-652 (1987); PCT Publication

NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res., 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5′-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded

hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5′-UG-3′. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5′ end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

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Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5′ Nucleotide of

the Clone Sequence and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5′ Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5′ Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5′ Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3′ Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization

conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table

1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a

nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence

selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 5, preferably excluded are one or more polynucleotides comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 5 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 5. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences referred to in the fifth column of Table 5. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 5

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
1	HWLFJ10	11	910143	AA922217, AW451106, AI949280, AW451993, AA454675, R26762, N35987, AA456121, AA204920, AI669723, R25954, and C02224.
1	HWLFJ10	39	907115	AA922217, AW451106, AI949280, AW451993, AA454675, R26762, N35987, AA456121, R25954, AA204920, AI669723, C02224, Z99396,

				AL134524, AL038837, AL037051, AL036725, AW372827, AA631969, AL039074, AL039440, AL119324, AL039085, AL039564, AL039156, AL039108, AL039109, AL039128, AL039659, AL038531, AL036924, AL039625, AL039648, AL045337, AW392670, AL037526, AL036858, AL039678, AL039629, AL037094, AL039150, AL039423, AL040992, AL042909, AL036238, AL037639, AL134110, AL036418, AL038447, AL037726, AL036196, AL039410, AL038509, AL036767, AL037082, AL036190, AL045353, AL038851, AL037615, AL036973, AL037077, AL036268, AL135012, AL044407, AL119457, AL036733, AL037027, AL119484, AL036679, AL119399, AL045327, AL119319, AL036998, AL045494, AL038520, AW384394, AL119522, AL042523, AL119391, AL037178, AL037085, AL119443, AW363220, AL036719, AL039386, AL119497, U46350, AL036191, AL037054, AL037021, AL036765, AL119439, AL039924, AL119363, AL119483, U46351, AL119355, AL119418, AL119335, AL042544, AL045328, U46349, U46341, AL042420, AL119341, AL042468, AL119396, U46347, AL119496, AL036158, AL134530, AL119444, AL037205, U46346, AL047163, AL036999, AL042614, AL036836, AL119401, AL134525, U46344, AI142139, AL045891, AL134519, AL119464, AL134518, AL044530, AL042655, AL134528, AL042741, AL042984, AL042965, AL042975, AI142137, U46345, AL134538, AL042542, AL043019, AL042898, AL043029, AL042551, AL042450, AL043003, AL036964, AL036886, AL036774, AL043321, AR066494, AR023813, AR060234, AR064707, A81671, AR069079, AR064706, A85203, Y11449, Y11447, AB026436, AL133053, AL122101, and AR054110.
2	HEEAM62	12	910116	AI821714, AI792133, AI791913, AA610491, AI821785, AW327868, AW410400, AW265385, AA714110, AI753113, AW327624, AA613203, AI797901, AI267818, AA526787, AI754653, AW302048, AI859946, AW270619, AI687343, AL079734, AI753488, AI612142, AA516047, AL120959, AI133164, AI801505, AL038705, AI732251, AC005722, AC004534, X54180, D83989, AF015157, AC004895, AL023803, AF077058, U95742, AC009178, AC002504, X75335, AC007216, AF001549, AC007790, AC003962, U91326, AC007030, AC006487, AL022326, AC004983, AC004858, AC005874, AF134471, AL034418, AC007226, AP000123, AC005037, AL008715, AC004778, AC005755, AP001054, AC005839, AC000102, AL049765, AC004517, AL080243, AL121655, AL034429, AF207550, AC007845, AC003963, U91322, AL049539, Z93023, AC002430, AC006211, AC005841, AC004687, AL031346, AL021707,

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2	HEEAM62	40	880094	AC005722.
2	HEEAM62	41	884825	AA610491, AI821714, AI792133, AI791913, AI821785, AW327868, AW410400, AW265385, AI753113, AW327624, AA714110, AI754653, AA613203, AI797901, AI267818, AW302048, AI859946, AA526787, AW270619, AL079734, AI753488, AI612142, AI687343, AW277253, AW438856, AA515728, AL120959, AI801505, AI133164, AC005722, AC004534, AC004895, D83989, X54180, AF015157, U91326, AC009178, U95742, AF077058, AL023803, AC007790, AC007216, AF001549, AL121825, AC003962, AC007030, AL022326, AC002504, AC004858, AC006211, AC007226, X75335, AF109907, AC006449, AL096701, AC002430, AC004885, AC006487, AC007384, AL034429, AL121655, AC004983, AC005874, AF134471, Z93023, AL034418, AC002553, AL022476, AP000123, AC005037, AL008715, L78833, AC002524, AC005280, L44140, AL035455, AC005839, AL021707, AC004963, AC002036, AC004517, AL049765, AC005746, AC005486, AL049795,

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2	HEEAM62	42	884394	AC005722.
3	HWLEC41	13	1016925	W16757, AA330909, AA464478, AA365797, AI890120, AA340214, AI708013, AA310896, AA374580, N59492, AA442510, AA641138, AA832085, AI904918, AA554571, AI521642, AA165705, AA515838, AA639767, AI860211, AA947454, H09071, AI671500, AI969288, AI636405, Y18417, AL136743, AF155138, AB024301, AF151804, AB013912, AC005668, AC008151, AC002044, AC008009, Z82214, AC006571, AC002314, AL022313, AC005519, AC006538, AC004914, AC004841, AC006275, AC004235, AC005391, AC005667, AP000359, AC007371, AC006277, AC008134, AC006211, AC007444, AC010206, AC005071, AC006480, AC005777, AC005756, AC005330, AC006285, AC005231, AC002565, AC007308, AC005778, AC006511, AC007298, AD000684, AL035450, AL133163, AL035587, AC006130, AL049569, AL022163, AL033525, AL022315, AC005072, AC004021, AC006101, AL021154, AC000090, AC005871, AC007773, AC004787, AL079301, AL022721, AC004675, AC005486, U80017,

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3	HWLEC41	43	889797	W16757, AA330909, AA464478, AA365797, AI890120, AA340214, AI708013, AA310896, AA374580, N59492, AA442510, AA641138, AI904918, AA554571, AI521642, AA832085, AA165705, AA515838, AA639767, AI860211, AW392670, AW363220, AL119522, AW372827, AL119319, AW384394, Z99396, U46351, AL119457, AL119324, AL119484, AL119483, AL119391, U46349, AL042975, AL119497, AL119443, AL119363, AL119355, AL134533, U46347, AL042965, AL119401, AL119396, U46350, AL119439, AL119341, AL042984, AI142139, AL119335, U46341, AL037205, AL043019, AL134527, AL119444, AL134531, AL042544, AL134536, AL119418, AL042614, AL043029, AL134525, AL042542, AL119496, U46346, AL043003, AL042433, AL134538, AL119399, AI142137, AL134920, AL134524, AL042450, U46345, AL134530, AL134519, AL042551, AL119464, AF155138, AB024301, AL136743, Y18417, AF151804, AB013912, AR060234, AR066494, A81671, AB026436, AR069079, and AR054110.

3	HWLEC41	44	778370	AA947454, H09071, AI671500, AI969288, AI636405, AI420028, AA983199, AL049834, AC006571, AC008009, AC008134, AC002044, AC006511, AC004841, AL022315, AC002299, AC007308, AC005667, AC006480, AL031466, AL080241, AC007934, AC002314, AC000026, AC002565, AL022163, AC002470, AL009179, AC005519, AC003006, AF047825, AC005993, AL080243, AC005072, AC008012, AC008042, AC004235, AC005007, AC006277, AB003151, AP000688, AC002059, AC005778, AC004787, AL033525, AL035587, AL117354, AC003070, AL022721, AC004083, AC007664, AC008018, AC006318, AL049539, AL078638, AC003682, Z82214, AC002352, AC006050, AC005274, AC004021, AC005391, AC006130, AC004985, AC010206, AL133448, AC005031, AC005668, AC006388, AL021155, AC004835, AC005330, AC009516, AC007845, AC007541, AC004467, AL031054, Z83844, AC005858, AC007773, AC007371, AC006285, AL035450, AL021368, AL049872, U80017, AC000134, AL034420, AC007364, AL049576, AC004825, AC005722, AL021154, AC004019, Z85987, AC005005, AC000090, Z97053, AC002096, AC004771, AF190465, AC005412, AC005057, AP000081, AC005229, AC004895, AC005484, AC005531, AL031005, AC006211, AL133163, AC005777, AF108083, AC002476, AP000359, AC004112, AC006275, AC005305, Z82203, AF196969, AC002420, AC004466, AC003085, AC004675, AC004885, AL031276, AC009263, AC006101, AF196779, AC006251, AC005808, X14448, AC005088, AC005304, AC005924, AC000025, AL022159, AC004491, AP000039, AF088219, AC005527, AP000326, AC005209, AC007919, AF053356, AL133312, AC004000, AC006111, AL031293, AC004195, AL035411, AP000697, AC009044, AL023879, AC003662, AL024498, Z73417, AP000054, AP000169, AP000704, AL022316, AF111168, AC005703, AC007055, AC006006, AC005944, AC007057, AL049569, AC004815, AP000211, AP000133, AF134726, AC005015, AC005387, AC007666, AC002558, AC004699, Z83845, AL031286, AC002554, AC004030, AL009051, Z93023, AC005841, AC006965, AL035422, AC003029, AC005255, AC005756, AC002430, AC006238, AC005003, AL022328, AC006538, AC006501, AC016025, AC005881, AP000557, AC006208, AC008122, AF001550, AC005500, and AL133353.
4	HWLFR02	14	908148	AI084806, AI750523, AI905553, AA037321, and AL110155.
4	HWLFR02	45	906666	AI084806, AI750523, AA037321, AI905553, AW392670, AW372827, AL134528, AL134524, AW363220, AW384394, AL134518, U46349, AL134902, AL119391, U46351, AL119484,

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4	HWLFR02	46	793707	AL134524, AL045327, AL134110, AL135012, AL038878, AL045328, U46344, AL045494, AL042523, AL047163, AL042898, AL042420, AI318479, AL037295, AL038838, AL037343, AI547295, AL038983, AI142134, AL042519, D29033, AL042468, AL037436, AL037335, AL048657, AL045891, AL038040, AL042741, AL042655, AL038761, AL037727, AL037443, AL038532, AI547258, AR066494, A93931, A85203, AL133053, AL122101, A93923, A93916, AL133074, AR023813, and AL133082.
5	HE9QN39	15	908124	AI902578, AI688582, AA777773, AW379424, AW369760, AA703884, AI907963, AI972020, AA916666, AW385387, AW385386, AI908566, AI356198, AA308054, N26808, AW152059, AI905077, AI694175, AW074054, AI985668, AI160378, AA937352, AA227090, AA494379, AA865313, AA506775, AA043012, AA612659, AI634278, AI632877, AI040102, AI872785, R91358, AA506766, AW084196, AI207271, R14440, AA226793, AA294950, AI678509, N39498, AA088691, AA323130, AI905195, AW380731, AA347026, H49126, AA337170, AA295197, AA304723, AL044263, AA332792, AI524587, AI423137, AI537066, AW071058, AI939550, C00596, T24948, AA223662, AA747032, AI336688, AA042886, AA506788, AW188897, AI205158, AA506790, AA625170, AI093888, AW385396, AA347025, AB033073, AL133001, and AL034418.
5	HE9QN39	47	907105	AI902578, AI688582, AW379424, AA777773, AW369760, AA703884, AI907963, AI423137, AI972020, AA916666, AW385387, AW385386, AW071058, AI908566, AI356198, AA308054, N26808, AA088691, AW152059, AI905077, AA424942, AI694175, AA747032, AW074054, AA625170, AI985668, AA937352, AI336688, AI160378, H49126, AA227090, AA494379, AA865313, AA506775, AA043012, AI093888, AA612659, AI040102, AI632877, AI872785, R91358, AA506766, AI634278, AW084196, AI205158, AA424941, AI207271, AA347025, R14440, AA294950, AA226793, N39498, AA323130, AI678509, AI824415, AA716420, AI905195, AW380731, AI207949, AA347026, AA337170, AA295197, AA304723, AL044263,

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6	HNHKL90	16	988941	AL042667, AL042670, AL041924, AI732720, AI753904, AI251696, AI568376, AI254267, AW192419, AI284543, AI090377, AW023975, AA631915, AW104161, AW270385, AW084445, AI254508, AI572680, AI251034, AA916430, AI251024, AI252005, AI254463, AA809125, AW303098, AA831426, AW026305, AI223626, AI421950, AI419337, AI923052, AW072963, AW167909, AI344906, AA526542, AI889995, AL038527, AI345394, AI361090, AI251284, AI251203, AI078409, AW069227, AA425283, AW237905, T07225, AW086291, AI254770, AI797998, AI537020, AI869797, H60912, AW409621, F35684, AW409626, AA610381, AL036949, AA530958, R59567, AA555232, AA199578, AA679946, AI249853, AI358928, AI890324, AI973173, AL046409, AL044701, R99532, AW408767, AW022796, AL042735, AI792092, AI821056, AI821805, AA278496, AI434653, AI732682, N92588, AI857834, AI590404, AA169245, AW389929, AI734119, AI625604, AA995373, AL009181, AL031577, AC005274, AC005412, AC007011, U52112, Z95116, AC007541, AC006208, AL133355, AP000030, AC005879, AL121754, AC002369, AP000553, AL021391, AC004805, AC002126, AP000152, AL049776, AC005206, Z96074, AL031432, AC007182, AL050321, AC005667, AC006538, AC004778, AC006064, AP000251, AP000555, Z95114, AC004383, AL022316, AC003042, Z84469, AF217403, U91318, AL080243, AC004583, U91324, AL132642, AL008719, AC003966, AC003101, AC005939, AL117338, Z98304, AC004448, AL008718, AC005751, AC005837, AC004832, AC004253, AL109613, Z93241, AC004834, AC005500, AC007934, U91322, AF134726, AC004797, AC004938, AC009516, AC002357, L44140, AL031228, AC005257, AC008018, AC005358, AC006353, Z93244, AL031311, AC005911, AL096829, AC007878, AL022336, AC005624, AC007731, AL031295, AC006241, AC004659, AP000008, AC006160, AC007664, AP000704, AC002054, AC005225, AC004126, AC006211, AC005231, L35532, AC005669, AL133396, AC005102, AL023575, AC005486, AF003626, AL133312, AC003049, AF129756, AL022726, Z93023, Y10196, Z98750, AP000557, AC005399, AC003089, AL117258, AC004854, U95739, AC004526, AL022333, AC005598, AC022517, AP000692, AP000014, AL021808, AL049836, AJ003147, AF109907, AL049569, Z86090,

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6	HNHKL90	48	886217	AA584484, F35684, AA610255, AI636734, AW273201, AW273146, AA558404, AA278496, AA557911, AA744094, AA857812, AI250552, AI251034, AI254770, AI284543, AI054090, AA410771, AW270385, AI572680, AW023975, AW237905, AA598605, AI869786, AI251284, AI251203, AI223626, AI249853, AL042667, AL042670, AA995373, AA599080, AI445373, AA778962, R67701, AA834817, AW303098, AI246061, AI340832, AA467740, AI923052, AW166641, H62550, H79586, AA565232, AA493789, AI251241, AA501867, AA678472, AA706156, AA302978, AW167909, AA371410, AI361090, AI419337, AA553457, H93152, AA831408, F32893, AI869797, AA297961, AA758244, AI468993, AA378474, AA182731, R99533, AI932871, AI732243, R83068, AA361513, AI620014, AA878344, T17332, AA569206, AI653776, AA084950, AI254267, AA744048, AI275183, F02437, AI888298, AI798313, AA135988, AA491722, AI279477, T03412, AA613761, AI190648, AA572983, AI251944, AI185394, AA074713, AA862312, AL120158, AW021116, AI358928, AA385775, AA593471, AI679045, AI421950, AL037777,

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6	HNHKL90	49	778375	AI908381, AW301855, AA134007, AL036949, AI908380, AA729004, AA661554, AI890283, AI752977, AA569206, AW023376, AI245693, AA446931, AI174827, AI982975, AW440424, AA847340, AW029626, R86735, AI888050, AA757646, AW080118, AI884404, AA664924, AA577719, R93642, AW149241, AI940546, AI969090, AA969564, AW022796, AA358531, AW020891, AA613189, N68191, AW081890, H77666, AI857834, AA730795, AI815770, AA774309, AC002094, M63480, M63544, AC022517, AC007919, AC005412, AC005200, AC005924, AC006441, AC005057, AC007687, AC004771, AL080245, AC005291, AC005399, AC007051, AC005779, AL121603, AC005914, U52112, AL080243, AC006449, AC005911, AC006162, Z69890, AC003102, AC004019, AL009179, AC002310, AC006511, AC004653, AC005839, AL109963, AL023575, AC005763, AL121658, AL035071, AC004953, AC005004, AC005519, AC005971, AF207550, AL096791, AC005562, AC005913, AC004659, AC006111, AC007686, AF111168, AC004144, AC000353, AC005527, AC002350, AP000555, Z98200, AC006057, AC002369, AC005944, AC002543, AC001228, AL008719, AC007285, AC006581, AC003030, AF139813, AC006285, Z85987, AC005565, AC004458, AL078460, AP000506, AB000882, Z81364, AC002301, AC003982, U91326, AC002544, AC005071, AC005940, AC006948, AC005081, U85195, AL049709, AL031685, AC006071, AC002126, AD000092, AC005529, AC005015, AC004549, AC007207, AP000280, AC004084, AB020865, AL109801, AE000658, AC003025, AC007565, AC004149, Z92542, Z84469, AL023807, AC004448, AC004816, AP000107, AP000039, U73627, AC016025, AL133355, AC005500, AC006166, AC004228, AF001552, AC002299, AL022322, AB015355, AC004884, AC005726, AC009405, AC006344, AP000106, AP000038, M63796, AL035658, AC007151, AL049843, AL022721, AC003041, AC004859, AC003098, AC004531, AC005484, AC005837, AC007546, AL031258, AC005907, AC005180, AC008116, AL031589, AC003047, AL050318, AC005826, AL121655, AL096808, AC000052, AC004253, AB023051, AC004038, AC007731, AC006379, AC005670, AL031295, AL008582, AC005589, AC002306, AL133246, Z93017, AC004851, AC004671, AC005696, AC007899, AF118808, AC009509, AC005274, AC004033, AP000497, AP000692,

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7	HSXEQ06	17	1016924	AI670834, AI793031, AA481590, AA884278, AI554009, AA418164, AI287582, N39228, AA177106, AA773834, AA233042, AI366763, AA417913, AA232936, AW085026, N35014, R46292, F12643, AA953139, Z43417, R54534, AI249382, AI817549, H53484, T34371, T32748, AI268132, H26220, F08007, AA976991, Z39490, N72112, F03527, F04961, AA357869, H99278, F10258, R81334, R37424, F07252, T74533, AA976568, R54437, AI433026, N46671, H13274, N46079, AA481525, D62268, AA360677, H08120, R66918, R76039, AA296802, W00375, N43768, H93364, N46077, AI559424, N72148, AA029181, AA743316, H08119, F36454, AA361446, T77292, W00419, AL135691, AA057340, H86888, AA044960, H38947, D20601, AW293865, and AF007142.
7	HSXEQ06	50	889664	AA481590, H53484, AA233042, F12643, Z43417, F08007, F07252, R54534, T74533, AA360677, W00375, AA296802, N43768, H08119, AA361446, AA953139, T77292, W00419, AL135691, AA057340, H86888, AA044960, H38947, AW293865, and AF007142.
7	HSXEQ06	51	895602	AI670834, AI793031, AA884278, AI554009, AA418164, AI287582, AA481590, AA177106, N39228, AA773834, AA233042, AI366763, AA417913, AA232936, AW085026, N35014, R46292, F12643, AA953139, R54534, Z43417, AI249382, T34371, T32748, AI268132, AI817549, H26220, F08007, AA976991, Z39490, N72112, F03527, F04961, AA357869, H99278, F10258, R37424, R81334, F07252, T74533, R54437, AA976568, AI433026, N46671, H13274, N46079, AA481525, D62268, H08120, R66918, R76039, AI559424, H93364, N46077, N72148, AA029181, H08119, AA743316, F36454, AA361446, AA296802, W00419, D20601, AA360677, H53484, and AF007142.
8	HEEAA16	18	977094	AW160964, AW161554, AA625323, AW161819, AW271229, AW339314, AW162568, AI885708, AW182755, AI879349, N21189, AW024687, AI422984, AA716508, AA524199, AI824115, AI039987, AI363318, AI144551, N32645, AI086639, N36662, AA452616, AA421616, N34750, AW172404, AI075719, AI879728, AA938122, AA423901, AA593691, AI361242, W19984, AA486284, AI279132, AI193615, AI291456, H98662, N27221, AA423861, AI088472, AA910342, AA932393, N23180, N23189, N32307, AA593305, N42095, AA574121, AI033661, AA628598, N33034, AI699457, AI041955, AI494625, AA037038, AA855017, AW008283, AA450002, AW161598, W03065, AA011407, AA573464, N99614, AA489305,

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8	HEEAA16	52	896114	AW160964, AW161554, AA625323, AW161819, AW339314, AW271229, AW162568, AI885708, AW182755, AI879349, N21189, AW024687, AI422984, AA716508, AA524199, AI824115, AI039987, AI363318, AI144551, N32645, AI086639, N36662, AA452616, AA421616, N34750, AW172404, AI075719, AI879728, AA938122, AA423901, AA593691, AA486284, AI361242, W19984, AI193615, AI291456, AI279132, H98662, N27221, AA423861, AI088472, AA910342, AA932393, N23180, N23189, N32307, AA593305, N42095, AA574121, AI033661, AA628598, N33034, AI699457, AI041955, AA037038, AI494625, AA855017, AW008283, AA450002, AW161598, W03065, AA011407, AA573464, AA489305, AI283275, AA994113, N99614, AA514943, AA639455, AW080959, N24684, AI346390, AI358548,

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9	HSPBY63	19	1009601	AI755214, AI754105, AW023111, AW237905, F35374, AI754567, AI279417, AA502532, AI792578, AA644090, AW020088, AW069227,

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9	HSPBY63	55	833958	AI755214, AI754105, AW023111, AW237905, AA502532, F35374, AL042667, AL042670, AI792578, AI431303, AI754567, AI279417, AW419262, AA644090, AW021569, AW338042, AI687343, AL042373, AW302711, AI612142, AW303196, H73550, AW274349, AA503019, AI267356, AW301350, AI369580, AI791211, AA654013, AI821947, AI610468, AI821714, AI792133, AI791913, AI380617, AI275982, AA640430, AA804943, AW020088, AA579179, AI681962, AI249688, N57681, AA470344, AI583252, AI040051, AA640410, AA410788, AW150226, AI267450, AI871954, AI821785, AA825827, AW072923, AI054419, AA483606, AL138182, AW275971, AI281730, AA714110, AA570740, AA829036, AW069227, T74524, AI887235, R97235, AA663461, AW131356, AI634187, AL042420, AI278972, AA719524, AI038304, H05940, AI590053, AI366993, AI590485, AW081303, AW303008, AA581525, AI053978, AW088049, AL038759, AI636734, AW021399, AI809818, H91062, AI569100, AI473943, AI049709, AI144081, T54146, AI358712, AA568204, AA847499, AI792304, AI523316, AI821200, AI440117, AW192599, AW407632, AA747757, AI733856, AA833875, AA833896, AI925588, AW271904, AC003982, AL021154, AL049694, AC004922, AL022316, AC000353, AC004526, AC005280, AC002316, AC005261, AC006487, AC006111, AC006011, AC005921, AC007934, AC006561, AL031846,

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10	HE8QV43	20	1034601	AI672059, AA431226, AW020448, AA931890, AI862704, AA432248, AI767980, AI080204, R61511, N70172, AI217478, H19344, R61567, H19054, AI767095, T16688, R07078, Z42320, H16566, H16608, AI984342, R12874, AW022003, AW382744, R41262, AW382729, AI041000, N98368, AI359566, AI361437, Z38551, R07033, and AA094554.
10	HE8QV43	56	889765	AA931890, R61567, H19054, H16608, R07078, Z42320, R12874, N98368, AW382744, AW382729, AA094554, and AW020448.
10	HE8QV43	57	897641	AI672059, AA431226, AI862704, AI767980, AA432248, R61511, AI080204, N70172, AW020448, AI217478, H19344, AI767095, H16566, T16688, AI984342, AW022003, R41262, AI041000, AI359566, AI361437, Z38551, and R07033.
10	HE8QV43	58	897640	AA931890, AW020448, AW382744, AW382729, AA431226, and AI672059.
11	HWLJX42	21	931868	AI373299, AA700893, AI401347, AI539297, AI017826, AI986324, AL121378, AA626076, AI261220, AI247251, F09841, T10264, H14604, AI002258, AI274645, AA092375, R07847, D12036, T66177, AI491842, AI932794, AI620639, AW149925, AW075484, AI677796, AI863382, AA449768, AL120254, AI633314, AI345347, AI886206, AI582483, AI554344, AW051088, AI866469, AI868931, AI469270, AI445990, AI251221, AI579901, AI538716, AW087207, AI582932, AI611738, AI589261, AI590227, AI335208, AI288305, AI571868, AI241923, AI580674, AI608936, AI613270, AI288285, AL042382, AI866770, AI955943, H89138, AW029186, AI925502, AL046942, AI784252, AI590630, AA983883, AI161279, AI335426, AI348777, AL041150, AI859464, AI345416, AI345612, AI439717, AI345415, AI865906, AI681985, AI819522, AI886123, AA470491, AW088328, AI961589, AW118518, AW168485, AI174394, AI432030, AL046595, AI570140, AI580436, AI280747, AI352326, AI951076, AI554821, AI439762, AL119863, AI624293, AI347854, AI933992, AI683173, AW008085, AW090393, AI923370, AI468872, AI872423, AI439452, AI670009, AI571909, AI073952, AI609069, AI630928, AI886181, AI475806, AW193026, AI868204, AW072719, AI798351, AI687127, AW023338, AI917252, AI682798, AI814087, AI783997, AI799183, AL079963, AW079075, AA225339, AI572717, AI862024, AW131282, AI628337, AI498067, AI700159, AI612750, AI590043, AW054931, AL037582, AL037602, AI690480, AI684244, AI800138, AI583558, AI494201, AI567612, AI859585, AI917963, AW117652, AI569583, AI627988,

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11	HWLJX42	59	889683	H14709, F12211, T10265, R07898, AL121378, AA700893, and AF200357.
11	HWLJX42	60	902376	AI373299, AA700893, AI401347, AI539297, AI017826, AI986324, AL121378, AA626076, AI261220, AI247251, F09841, T10264, H14604, AI002258, AI274645, AA092375, R07847, D12036, T66177, AI719118, and AF200357.
12	HAPSO15	22	998849	AI005338, AI990295, AA614439, AI653319, AI765704, AI160949, W94185, AI697154, AW235878, AA291173, AI090634, AW293238, AW136705, AA344126, AI825396, R26919, AA838408, T97232, AI829192, AI699605, AI768022, AI433590, AI569328, AL046466, AI358042, AI648408, AI863321, AW189415, AI280661, AI537617, AI249877, AI758528, AI500061, AI446809, W22165, AL041573, AI612759, AI249962, AI432040, AI281867, AI540784, AI470293, AW192461, AI648684, AI345416, AI345612, AW152182, AI345415, AI445430, AI687166, AI310332, AI471227, AI824576, AI445588, F27788, AW162189, AI624548, AI889932, AW163834, AL040241, AI874166, AI926794, AW169848, AA449768, AI540458, AI619502, AI590686, AI423105, AI679098, AL039086, AA437338, AW170725, AI689420, AL042745, AI890907, AI798351, AI923357, AA225339, AI866127, AI269696, AL046944, AI472566, AI241923, AI919345, AW167918, AI682652, AI159837, AI884318,

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12	HAPSO15	61	907116	AI005338, AI990295, AA614439, AI653319, AI765704, AI160949, W94185, AI697154, AW235878, AA291173, AI090634, AW293238, AW136705, AA344126, AI825396, R26919, AA838408, T97232, AI829192, AI699605, AI768022, AI433590, AI569328, AL046466, AI358042, AI648408, AI863321, AW189415, AI280661, AI537617, AI249877, AI758528, AI500061, AI446809, W22165, AL041573, AI612759, AI249962, AI432040, AI281867, AI540784, AI470293, AW192461, AI648684, AI345416, AI345612, AW152182, AI345415, AI445430, AI687166, AI310332, AI824576, AI471227, AI445588, F27788, AW162189,

				AI624548, AI889932, AW163834, AL040241, AI874166, AI926794, AW169848, AA449768, AI540458, AI619502, AI590686, AI423105, AI679098, AL039086, AA437338, AL042745, AI689420, AW170725, AI890907, AI798351, AI923357, AA225339, AI866127, C14331, AI269696, AL046944, AI241923, AI472566, AI919345, AW167918, AI682652, AI159837, AI884318, AW129929, AI874261, AI445992, AI699011, AI560023, AI446248, AL134999, AI469505, AW302992, AI758735, AI348917, AW195969, AI800380, AL037454, AW167086, F37471, AI349957, AW021717, AI582912, AI345005, AI570966, AI335208, AI860897, AI366900, AI699865, AL036638, AW169604, AI670009, AI921254, AW079572, AI648509, AI536664, AI541056, AI890507, AI863014, AL079963, AI683559, AI697092, AW193467, AI522052, AW162194, AI590043, AA580663, AI335426, AI348777, AI866770, AA641818, AI567582, AI801793, AL038564, AI679321, AI671679, AI249946, AI499285, AI568138, AI922550, AL048644, AI434741, AI690813, AI499621, AL047187, AW088628, AI648567, AI921176, AW088691, AI671642, AI955906, AW022699, AI537024, AI798101, AW089275, AI687065, AL036673, AI318569, AW130403, AI933574, AW102861, AW083374, AI627988, N71199, AI922365, AI698391, AL037582, AL037602, AI500714, AI589668, AI345608, AI890183, AI445990, AW104056, AI476046, AL047763, AI572717, AI611810, AW079409, AI620284, AA805434, AI932949, AI685080, AL041150, AW131999, AI434038, AI273964, AI886594, AI620302, AI687134, AI089782, AI345677, AI553645, AI636588, AW028416, AI280732, AI280521, AI927755, AI349645, AI344785, AW189424, AL040449, AW083804, AI306705, AL042744, AI345471, AI627893, AW163554, AI874228, AW268060, AI308035, AL119863, AI336575, AA291456, AI291601, AI499393, AI254814, AL121014, AI612015, AI348897, F35653, AI334450, AI811344, AW161202, AW302973, AA284380, N29277, AI590415, AI612750, AI679672; AI307494, AI310575, AW262565, AL046595, AI624529, AI251221, AI554343, AI446373, AW020419, AI628015, AW087907, AI349622, AI267162, AA420722, AW268220, AL034582, Z56887, AF176117, AF176116, Z82022, AR018138, AF183393, AL049382, AL080154, A62298, I48978, AL050116, AF078844, X63410, AL137550, AF215669, AJ012755, I89947, X82434, AR038854, L19437, A08916, AF090934, S68736, AL117416, A08913, A84916, AF061981, A18777, AL122106, A30438, A08912, A08910, A08909, AF079763, AF090943, A08908, X80340,

				AL133010, AF061943, A15345, AL137276, AF036941, AR013797, X84990, AF000301, AL137488, AL050138, AL137548, A03736, AR020905, I89931, AB019565, I49625, I48979, E02221, X53587, AF026816, AL133098, AL050149, AF058921, AF090901, AF106657, AL080148, AL137521, Y11587, AF113699, AF120268, AF113690, AF176651, AB007812, E01314, AR011880, I41145, X00861, AF028823, AL133558, AL110280, S76508, AL137271, AF017437, X52128, AL080127, S77771, A65340, Y11254, AR000496, E06743, U39656, AL049452, AF106862, AL137533, AF030513, AF113691, AL080060, U88966, AL133113, S75997, AF113694, AL137429, AL133067, AF113677, Y16645, Y10823, A77033, A77035, AL049314, E02349, AF159615, AL117460, AF106697, AL137574, S69510, AL133014, U78525, AL096751, AF113013, U35846, AF162270, X87582, Z72491, AF111851, AF125948, AF097996, AL137479, I89934, I89944, AR029490, U58996, AL117583, S79832, AL080126, AF022363, E04233, A93350, AF047716, AF057299, AL110171, AF067728, AL117585, AL137529, AL110221, M27260, AF090903, AL133016, AL137547, AL122118, S78214, AF067790, AF091084, AF003737, AF113019, E03348, AF113689, S36676, A65341, E02253, A83556, E03349, AF087943, I33392, AF118090, L30117, AR059958, I17544, AL117649, AF026124, AL117578, U87620, Y14314, AF158248, AL117440, AL137665, AL080163, A58524, A08911, A58523, A21103, AL137556, AF126247, AF118094, AL137557, X79812, AF192557, AL137459, AJ238278, AL080234, AL133075, AF090900, AL080140, AL122093, U42766, AF102578, X96540, I68732, X72889, AF111112, AL050277, AL080074, A08907, U95114, U00763, E05822, I03321, AL133557, AL080158, AF061795, AL050155, AF151685, AL050146, A23630, A08915, X06146, Y10655, AL133093, AL050024, U67958, AF132676, AL133640, AF061836, AL050172, AL122098, D16301, Y09972, AL122123, E12747, A18788, X81464, U75932, S61953, AL122110, AF118070, AL137478, AL080159, AL137560, Z37987, E07108, AF205861, I09499, AL137558, A45787, AF185576, AF146568, U80742, E15569, A52563, AL137527, AF139986, AL050092, and AA251086.
12	HAPSO15	62	861319	AL043064, AL043063, AI660939, AI985121, AI709336, AA195089, AI651665, AI334303, AW166009, AI419816, AW264384, AI417989, AI283117, AW118060, T59567, AI469990, AI805955, AI417034, AL040305, AA259095, AA476494, AA032213, AI299782, AI360924, AI992080, AA160957, AA449165, AI074310,

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13	HE8QG24	23	1043448	R19968, R45070, R45070, H18507, H19173, H23114, H23115, AA284156, AA291235, AA825831, AA598556, Z38807, Z42645, AI204609, AI288225, and AW027842.
13	HE8QG24	63	880107	H23114, H19173, Z42645, and R19968.
13	HE8QG24	64	834909	AI809248, AA291235, AA825831, AW182399, AI288225, AA598556, AI204609, AL044143, H23115, H18507, R45070, Z38807, AA362871, AA284156, and AW027842.
14	HCRNO87	24	1035379	AA601278, AA572968, AL044339, AL079734, AI358712, AL041924, AL042906, AL042905, AW304580, AI282253, AI254959, AI345334, AW022934, AW023111, AA515728, AI440117, AA744021, AI815583, AI816537, AA502532, AA644090, AW237905, AL042539, AI049709, AW157180, AA013168, AL042667, AL042670, AI754170, AI279402, AA019973, AA483606, AI635440, AA570740, AA706495, AA584655, AA530958, AI431513, AW270385, AW069227, AA568204, AW029515, AI792439, AI369580, AL079894, AI859438, AW021161, AA535216, AI733856, AI080732, AA313025, AI583142, AI923052, AA877992, AI189348, AA807704, AA683069, AA282951, H82636, AA775205, AA604843, AW275971, AA491767, AI610468, AA284247, AA225890, AI345497, AI754336, AW069412, AI565138, AA323343, AA158549, AI887235, AA602906, H63660, AA054085, AA832145, AA634071, AI635609, AI536834, AI223968, AI192440, AA149033, AA533054, AW275432, AA715814, AA484892, AA148197, AA805044, R99034, AI821382, AA129746, AI081147, AI457389, AI187148, AL040374,

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14	HCRNO87	65	875288	AW392670, AW363220, AW372827, AW384394, AL119497, Z99396, AL042965, AL119319, U46341, AL119457, AL119324, AL119363, AL119484, AL119341, AL119391, AL119355, AL119483, AL119443, AL119496, AL119522, AL119396, U46351, U46349, AL134538, AL119335, U46346, U46350, U46347, AL119418, AL119444, AL042975, AL134533, AL042614, AL037205, AL134920, AL119439, AL043029, AL134532, AL134528, AL134531, AL119399, AL134518, U46345, AL042984, AL042970, AL042450, AL042542, AL043011, AL042544, AL043019, AL042551, AL119464, AL119488, AL043003, A81671, AR060234, AR066494, AB026436, AR054110, and AR069079.
14	HCRNO87	67	793776	AI282253, AI912496, AL042377, AL079447, AI111171, AL048482, AI908996, AL042694, AI683483, AL042832, AL110373, AL045943, AI561147, AL046021, AI250821, AI921406, AI500711, AL138351, AA524800, AI242505, AW276792, AC004878, AL109628, AC002302, AL109758, AL035458, AF045555, Z98036, AC009501, AL121825, AC005874, AF134471, AC007842, AF001548, AL137627, AL132985, AC007371, AL034417, AC004686, AC006530, AC002381, AC004084, AC002551, AC003663, AL009051, AL096751, AL031984, AC006211, AC005071, AC004213, AC002086, Z83826, AC006288, AC006396, AL079342, AF001549, AC005409, AP000130, AP000208, AC007225, AC007263, AP000247, AC003101, AL096701, and AL022322.
15	HBKED12	68	843529	AA830149, M85983, AA017704, and AB014550.
15	HBKED12	69	892327	AW387461, AA452950, AW387390, AW387345, T49188, T49196, AW387357, AW387403, AL045456, AA775950, AI373679, AB014550, and AL080138.
15	HBKED12	70	799625	AL043732, AI373679, Z45113, AW387461, AW387390, AW387357, AW387403, AW387345, AA129303, R30697, AL080138, and AB014550.
15	HBKED12	71	799500	AL043732, AA129303, AA868754, AW182285, AI187414, AI025779, AW103175, AI439050, R30697, AI739367, AA730052, AI373679, AA905494, Z45113, AW387461, AW387357, AW387390, AW387403, AW387345, D62970, AL080138, and AB014550.

16	HE8UY36	26	961223	AL118635, AL118653, AW070370, AA731719, AA256355, AA687307, AA436385, AI672649, W56461, AA460908, AA573980, AI801382, AI356922, AA576483, N26544, N35657, AA680355, AI453021, AA931279, AA461210, D53983, T08585, AA216553, R44805, AI700161, M79189, Z43786, AA132512, R19140, AA361663, AA436687, R55567, AA814168, T30241, F11446, AA907822, AA256260, T30648, Z39853, AA779544, AA234808, Z44615, F09108, R55328, R40252, F03659, D53850, D54353, AW131701, AI868112, R14301, N55319, and AA234734.
16	HE8UY36	72	906497	AL118635, AL118653, AW070370, AA731719, AA256355, AA687307, AA436385, AI672649, W56461, AA460908, AA573980, AI801382, AI356922, N26544, AA576483, N35657, AA680355, AI453021, AA931279, D53983, T08585, AA461210, AA216553, R44805, AI700161, M79189, Z43786, AA132512, R19140, AA361663, AA436687, AA814168, T30241, R55567, F11446, AA907822, AA256260, T30648, Z39853, AA779544, AA234808, F09108, R55328, Z44615, R40252, F03659, D53850, D54353, AW131701, AI868112, R14301, N55319, AA234734, C14322, C14391, AJ239466, D51002, D81306, C14723, AI525556, AI535660, AI541205, D50992, AI525852, AI557312, AI536138, AI557084, AI540903, AI525500, AI541365, AI524961, AI557258, AI535639, AI541034, AI557533, H65400, AA058620, AI525757, AI547071, AI557602, AI525302, AI541075, AI557082, N71206, AI546829, AI557731, AI557474, AI541321, AI557238, AI535813, AI541154, AI557541, AI540974, AI525666, AI557543, Z33585, Z30183, AR050070, A62298, A82595, A82593, U94592, and U45328.
17	HNHNT13	27	1009532	T90906, T91278, AW090778, AL031320, AL020997, AL117344, AF045555, AC002982, AC004088, AL049793, AL031595, AL133216, Y12508, AP000348, AC005081, and AC004991.
18	HODEB50	76	906592	AA585325, D61185, T18597, Z32887, D59751, AA585098, R29657, AI525316, AI525500, C15406, AA585101, D57491, AI526078, Z32822, Z33559, D53161, R45895, AI557262, AI541535, AA585439, AI535660, AI526016, R28892, R28965, AI526140, AI557809, AI546971, AI541365, AI541517, AI541205, AI526169, AI557740, AI525556, AI541034, AI557718, AI557533, C14210, D54897, R28735, R29445, D60765, D60844, AI546831, AI526112, AI557810, AI526024, AA585356, AI526158, AI557408, AI557602, AI557727, AI526184, AI557763, AI526109, C16294, C16315, AI547250, AI557787, AI546999, AI525114, AI546875, AI541346, AI557758, AI557852, AI541013, R29218, AI547039, R28967, AI557734, AI541422, AI541374, AI541515, AI541492, AI546829,

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19	HWLFQ64	29	1035384	AA731688, AI538777, N50646, AL047042, AI349772, AI815383, AI906328, AW080838, AL119049, AL121270, AW071349, AW166645, AI907070, AL045500, AI349645, AI207510, AI868831, AW268253, AW162071, AL120854, AI149592, AW132121, AI624859, AI500553, AL036396, AI580190, AI349614, AL135661, AI064830, AI907061, AI436456, AI340582, AL036802, AL046849, AI220734, AI345111, AI349598, AI687376, AI344182, AI909662, AI690751, AI909666, AI920968, AA613907, AW303152, AI343112, AI433976, AI345860, AI433157, AL036146, AI251485, AL036759, AL120736, AI684265, AI863014, AW117882, AI682106, AL119748, AI813914, AI500077, AI334902, AL047763, AI475371, AI521012, AL119791, AL040169, AW238730, AI309401, AA528822, AI469532, AI679724, AI687415, AI608667, AI349933, AW302965, AW301409, AI590482, AL121365, AI567351, AI799305, AI560012, AI934036, AI687728, AI969601, AI568870, AI538716, AL036274, AW071417, AI907056, AI345744, AA528491, AL040243, AL038778, AI702406, AI969567, AI349256, AA640779, AI349937, AI343059, AI312152, AI525064, AI440426, AI567632, AI673256, AW089572, AI873731, AW074993, AW103371, AI285735, AL036980, AW268768, AI753683, AI818683, AI499393, AA603930, AA585422, AI349004, AL036240, AW168591, AI631107, AI547175, AI889203, AI758437, AL042382, AI282655, AI636456, AI609592, AI307466, AL038605, AI027531, AI682743, AI612913, AI345735, AI697137, AI690835, AI583316, AI366991, AI281779, AW235035, AW104724, AI686926, AW274192, AW087445, AW195957, AI635461, AI440239, AI250293, AI678302, AI671679, AI590128, AI635942, AI275175, AI497733, AA572758, AI366549, AI564719, AI340519, AI620284, AA938383, AI597918, AI699857, AI445432, AI625079, AI439087, AI866608, AI524991, AL048871, AI696846, AW169653, AW068845, AI919058, AI568854, AI866780, AI610307, AI857296, AI348897, AI886532, AW148320, AI498579, AI282903, AI536685, AI802542, AI446606, AI307558,

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19	HWLFQ64	77	889696	AA731688, AI538777, N50646, AL119457, AL119399, AL042382, AL042544, AL079794, AL079741, AL119324, AL119511, AI802542, AL041573, AI539771, AI269862, AI648663, AI702073, AL037081, AI559296, AI824746, AI569583, AI468872, AL121463, AL079963, AW238730, AI619502, AI433976, AI564719, AW088903, AI677796, AI497733, AI499285, AI934011, AI868831, AI433157, AI247293, AA470491, AL045500, AI889376, AI784252, AI524671, AI598061, AI571909, AI568296, AI922901, AW026882, AI648684, AW190042, AW149311, AW051258, AI345551, AI537677, AI862144, AI799470, AI610645, AI866608, AI340603, AI432969, AL036736, AW301505, AI680165, AI886124, AI926790, AL036802, AI648509, AL041772, AI921248, AI634345, AW262565, AI687065, AI801766, AW169653, AI536638, AL119863, AI890833, AI491852, AI590120, AA225339, AI828731, AI434223, AI284131, AW088134, AI439256, AI554245, AW081255, AL134830, AI800453, AI252023, AL036403, AI284517, AI633419, AI439745, AL119791, AI251830, AI812107, AI554427, AI349772, AI612759, AI871697, AW403717, AW302965, AA640779, AA287231, AL036980, AW071417, AI819326, AL040243, AA572758, AW163464, AI537024, AI886753, AI635461, AI587143, AI590227, AI921176, AI340519, AI498579, AA427700, AI684234, AI567128, AI679321, AI815232, AI612885, AA494167, AI445414, AI340582, AI800433, AW268220, AW129202, AI475371, AI609375, AI445165, AI873604, AI491775, AW149227, AI828682, AI282326, AW023590, AI567993, AI627360, AI312428, AI801325, AI590601, AI888953, AI250663, AL042551, AI923989, AI866002, AI923768, AI818977, AI343059, AI633125, AW148363, AI630928, AW161579, AI584140, AI536685, AI619748, AW166970, AI637584, AW080327, AI500523, AL036396, AI349933, AW059837, AI680498, AI919345, AI364788, AI611738, AI348897, AI308032, AI888501, AW166583, AI366549, AI811344, AI520785, AI636719, AI591316, AI539153, AI627988, AW302988, AI620284, AW104724, AI280661, AI500706, AI632408, AI582558, AW166903, AI567612, AL036274, AI696626, AI254731, AI567846, AI612913, AW132056, AW198090, AI862139, AL036214, AI446373, AW161156, AL039086, AI608936, AW148536, AI538085, AW086113, AI207510, AL047763, AI445025, AI554186, AI818683, AI224992, AI690426, AI783504, AI440239, AI636585, AI274013, AI521012, AI349645, AW074869, AI887396,

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20	HODFW41	30	1024938	R78575 and R78576.
20	HODFW41	78	833419	R78575 and R78576.
21	HE9RO44	31	963273	AA258202, AI797125, AA354717, AI695030, Z28688, Z24983, R02527, AI627534, AI767477, AB023179, and AL137715.
21	HE9RO44	79	886421	Z28688, Z24983, D51799, C14389, D59502, D80195, D80164, D81026, C15076, D80038, D59467, D59275, D80227, D58283, D80022, D80166, D80193, D59859, D59619, D80210, D80391, C14331, D80240, D80045, D59787, D51423, D81030, D80253, D80043, AA305578, D80269, D80212, D80196, D80188, D50979, D80219, AA514188, D59927, D57483, AA305409, D59610, D80378, D80366, D51022, D59889, D50995, D80024, AW360811, D80248, D80522, AW177440, D51060, D80251, D80241, C14014, AA514186, D80133, C14429, AW178893, AW375405, D80302, T03269, AW179328, AW366296, AW377671, AW360844, AW360817, C75259, AW375406, AW378534, AW179332, AW377672, AW179023, AW178905, D80268, AW378532, C05695, AW177501, AW177511, D80439, AW352171, AW377676, AW352170, AW177731, D80247, AW178907, AW178762, AW179019, AW179024, D51250, D80132, AW177505, AW178906, D59373, AW360841, AW179020, AW178775, AW178909, AW177456, AW179329, D80134, AW178980, AW177733, AW378528, AW178908, AW178754, AW179018, AW352158, D58253, AW352117, AW369651, AW176467, AW352174, D51103, D59695, AW179004, AW179012, AW178914, AW378525, D80157, AI910186, D52291, F13647, AW177728, D80064, T11417, AW367967, D59503, AW179009, D51759, C06015, AW178774, AW178911, D80949, AW378543, AW177722, AW352163, D80168, D58246, C14227, AI905856, AW178983, D81111, AW178781, C14298, T48593, C14077, C14407, AW360834, D45260, D59627, D59653, AW177723, AW352120, AW378540, D80258, D58101, Z21582, H67866, AW367950, AI525923, H67854, C03092, T03116, AA809122, D59317, AW378533, AI535850, C14975, D51097, AA285331, AW177508, AW178986, AI535686, D59551, AI525917, D51213, AW177497, D80014, D45273, C14973, AW177734, AI525920, C14344, D51221,

				AI557751, D59474, D60010, D60214, AA514184, AI525227, C14046, AI557774, C14957, AI525235, AI525925, AI525242, T02868, T03048, AI525215, D59976, AI525912, AW378542, AW378539, C16955, C05763, Z33452, T02974, AI525237, H67858, AI525222, AI525928, C13958, AW360855, AI535665, AB023179, AB028859, A84916, AJ132110, A62300, A62298, AR018138, AR008278, AF058696, A82595, X67155, Y17188, D26022, Y12724, A25909, A67220, D89785, A78862, D34614, AR060385, AB002449, D88547, A94995, X82626, AR008443, I82448, AR016808, I50126, I50132, I50128, I50133, AR025207, AR066488, AR016514, AR060138, A45456, A26615, AR052274, Y09669, A43192, A43190, AR038669, I14842, AR066487, AR066490, A30438, AR054175, I18367, AB012117, D50010, Y17187, A63261, AR008277, AR008281, A70867, AR008408, X68127, A85396, AR062872, D88507, AR066482, A44171, AR016691, AR016690, U46128, A85477, I19525, A86792, D13509, I79511, A64136, A68321, AR060133, X93549, U79457, AF123263, AR032065, and AR008382.
21	HE9RO44	80	834967	AI797125, AA354717, AI695030, AI627534, AI767477, AB023179, and AL137715.
22	HE9SE18	81	844163	AI469599, AA515728, AW157731, AI284126, C06142, AI499954, AW419389, AI369580, AI439393, AI633386, AI798407, F30158, AW304580, AI276298, AL043676, Z83838, AB026898, AL079342, AL022476, AL031311, AC012384, AL121754, AC007011, AL031650, AC003108, AP000493, AC006071, AC006120, AC003071, AC005071, AC002429, Z98304, AC000003, AL031427, AC005740, AC004491, AC004106, U91322, AC004590, Z94056, AF043945, AC005070, AC006121, AC005548, AL035588, AL031291, X87344, AC003098, AC004973, AL049591, Z95113, AC002492, AC006965, AC008012, AF196779, AC006512, AF111169, AC007040, AP000350, AC005500, AF196970, AC003663, AL031848, AC006064, Z95116, AC006450, AC004020, AC002416, AL049646, Z83822, AC005539, AC007151, AC004033, AP000501, AF124730, AC008126, AC007386, AL133399, AC004668, AP000315, AC007225, AL035427, AL049869, AP000702, AC004815, AL034417, AL035695, AP000165, AP000118, AC007216, AC006057, AL133500, AC004773, AC005288, AP000500, AC005988, AC005962, AL121578, AC005696, AC008085, AC004782, AC005291, AC006379, U47924, AC002412, AC004552, AL024507, AL133243, Z84469, AL031584, AL035684, AC006544, Z93783, AC006257, AL031846, Z98751, AC003010, AC005736, AL022165, AC004526, Z94801, AL080243, AF130342, Z84480, AL033527, U91323, AC007157, AC000385,

				AP000692, AC009510, AC002553, AL033521, AC005146, AC009247, AC005562, AF031078, AC004659, AC004963, AF029308, AC004841, AF030876, AC002420, AL133289, AC004765, AC010205, AC007172, AL049832, AC002302, AC007934, AC005682, AC007546, AC002310, AC008151, AL121852, AC007955, AL023575, AL117258, AL031657, AC007021, AC004796, AF205588, AC002301, AL022318, AL079295, Z84484, and AL022345.
22	HE9SE18	82	847705	AI053588, AW440273, AA630098, AI611561, AI753542, AI792443, AW276682, AI207861, AI053773, AI251700, AI251385, AA483217, AI400721, AW303037, AI254627, AW086339, W02028, AW302750, AW302739, AI254684, AI053963, AI419982, AI252712, AA250763, AW302730, AW303221, AA668673, AA191610, AI311626, AA703680, AI253208, AW262442, AW148392, AW085628, AI254217, AW271017, AI344886, AW183037, AW302803, AI252858, AW148344, AW268777, AA223924, H80554, AA206026, AL041838, AI493025, AA931216, AA885499, AI991553, AI559284, AW170681, AI935032, AW134612, AA664331, AA528253, AW268767, AW302321, AL121877, AF107257, AC007221, AC007193, AC004914, AL034406, AC000029, AL021808, AL049692, AC005303, Z83819, AC002302, AL035668, U85195, AE000658, AC007542, Z84466, AL008710, AC005803, AC006115, AL022101, AP000108, AP000040, AP000095, AC005538, AP000239, Z82198, AC005548, AC004934, AC007688, Z77249, AL033533, AC008012, AC006210, AC005875, AC002300, AJ239322, AC007159, Z96074, AC006600, AC011198, AC002080, AC005886, AF001549, AL132642, AL008718, AC003670, AC005913, U82757, AC007388, AC002462, AC005529, AJ239318, AC003684, AC005060, AC007298, AL031681, AJ003147, Z85996, AC005082, AC006380, AC007563, AC005799, AL035686, U52112, AP000525, AC005015, AL031118, AP000946, Z99289, Z97353, AF155238, AC004000, Z98304, U95743, AC004125, AC006949, AL023653, AC005277, AC007278, Z82189, AL049198, AC005553, AL050404, AC006021, AC004876, AL109654, AC002543, AL137100, AC005184, AP000066, AL023513, AP000247, U82668, AP000104, AP000236, AL031655, AC006042, AC004147, AL096791, AL049781, AC006538, AC004953, AL022330, AL022574, AC004910, AC004383, Z94057, AP000130, AP000208, AC005181, AC004962, AF133093, AF091512, AL135744, AC003663, AC004032, AC005137, AC007919, AP000697, AC005224, Y18000, AC007189, AC004929, AC003969, AL021918, AC005216, AC004015, AP000402, and AC004827.

23	HISCV60	33	908129	AA460355, Z84466, AC005015, U91326, AP000501, AL035420, AC004990, AL139054, AC004098, Z82244, AC006430, Z82178, AL022315, AC004983, AF051976, AC002070, AC005067, AC002470, AC007011, AC006441, AC002544, AC005049, AL049553, AC002045, AC005031, AL020997, AC002400, AL049832, AC005578, AF111168, AC007308, AC002425, AC008372, AL121652, Z82194, Z95116, AC007227, AC005387, AL135960, AJ131016, AC005833, AC007014, AL031311, U91325, AC004585, U91321, AC007263, AC005697, AC002352, AC007688, Z99128, AC007030, AL133163, AC005193, Z84469, AC000353, AC004662, AL049611, AC004381, AC006387, AL021397, AL022329, AC007664, AL035405, AL133245, AL035091, AC004656, U15177, Z98051, Y14768, L78810, AL008721, AC000086, AL031591, and AL050332.
23	HISCV60	83	906662	AA460355, Z84466, AP000501, AL139054, AL035420, AC004098, Z82178, AC005049, AC004983, AF051976, AL022315, AC002070, AC007011, AC006441, AL049553, AC005067, AC007308, AC002470, AC002544, AC002045, AL049832, AL121652, AL020997, AC005578, AC002425, AC008372, AC007014, AC005387, Z82194, AF111168, Z95116, AC002365, U15177, AL135960, AJ131016, AC007227, AC005833, AC007263, AC004585, AL031311, AC007688, AC005193, AC007664, U91321, Z99128, AL049611, Y14768, AC004656, AL133163, AC007030, AL031591, AL035091, L78810, AC006387, Z84469, AP000695, W27854, and R10707.
24	HNGOI12	84	838184	AC003675, AC005950, AC001228, and AJ006345.
24	HNGOI12	85	839283	AJ006345, AC005950, AC003675, and AC001228.
25	HE8UT25	35	1036044	AI565777, AA633940, AA486168, AA715046, AA769495, N79867, AI821692, AA443915, AA484716, AA505112, AA363854, AA857203, AI992118, R46645, AA707785, AI147350, N35484, AI510701, AA400248, W58074, AA740366, AA573765, AA927511, AA862943, AA490068, AI797190, Z33559, M78360, AA722705, AI148034, T95584, T99920, AA486328, N87913, R85520, AI535639, L48842, AI557262, AI535660, AI536138, R42423, AA663248, AI525556, AI541205, AI374775, AI541365, AI557731, D57491, AA585101, AA585439, AI557084, AI525316, T18597, AI541535, T66941, Z28355, R45895, R28735, AW043894, AA487956, AA400552, R29445, T11028, D61254, D61185, AI526140, AA093450, AI541346, AI557808, D55233, AI546875, AI557740, R29218, C16300, AI557807, T51773, R38570, AI546999, AI915048, AI525431,

				AI525306, Z32822, AI541374, AI546829, AA643967, AI557734, C16305, R28967, Z99297, Z97205, AC011456, AC005252, AC006002, Z54335, Z93931, AL034402, AC004928, AL031736, AC005181, AC000119, AL109760, AC004691, Z86064, AC004896, Z95889, AL049767, AC006600, AP000240, AC006257, AC004999, AL078474, Z99497, AP000040, AC004074, AF131798, AL049874, AC005908, AC005919, AL021877, AL050308, AC004070, AC005922, AC005323, AJ229041, AJ006998, AL121840, AC002288, AC010209, AP000108, AP000014, AL078583, AC007559, AC004029, AC004075, AL023755, L81803, AL049570, AP000158, AP000247, AL031431, AF205588, AL117337, AC007677, AC002366, AF099810, AC007630, AC008273, AP000282, AC007250, AC005988, AC004885, AC007225, AC007092, AC008127, AC007347, AP000096, AC009028, AC008085, AL031904, AL031732, AC006226, Z82190, AC004478, AF015720, AC006369, AP000432, AC006536, AC005002, AL079352, AC007284, AP000566, AL049735, AC005230, AC004976, AC006961, AC005684, AC004609, AC004601, AL078612, AC006373, AC004695, AL109963, AL132796, AL133512, AC005572, AC006354, AL121838, AL031737, AP000208, AP000130, AC004506, AB020868, AC004806, AC008069, AL109657, AC004905, AL035684, Z84467, AC008045, AC004953, AC008170, AL035251, AL035468, AJ229042, AC005160, AF051934, AC008929, AF130342, AL078622, AL022158, AC006032, AC004215, AL024458, AC004002, AC005265, AP000225, AC007102, AP000959, AC006368, AP000069, AL079303, AC007541, AL079340, AC002386, AC007590, AC004972, AC002990, AL050334, AF064865, AL034411, AC006044, AF129107, AC002045, AC003686, AC002070, AC006052, AL096757, AL034548, AF064862, AJ243213, AC004963, AP000086, AL033392, AC000392, AL132800, AC002981, AB000882, AC008498, AC006029, AC002349, AC007671, AL049867, AC005021, AP000224, AB020863, AC002494, and AC005863.
25	HE8UT25	87	834928	AI565777, W58074, AA715046, AA769495, AI821692, AA443915, AA484716, AA486168, AA633940, AA505112, AA857203, AI992118, AA707785, AI147350, N35484, AI510701, AA400248, AA740366, R46645, AA363854, N79867, AA573765, AA927511, AA490068, AA722705, T95584, T99920, AI148034, AA862943, W37866, AI797190, R42423, AA486328, R85520, AA663248, AI374775, AI280238, AI148165, T66941, AL045187, L48842, AA487956, AW043894, AA581070, AA400552, AI114816, AW016892, AI915048, AA643967,

				T51773, AI918922, AI609923, R38570, AW248583, N87913, R84390, AF131798, Z82190, Z99297, AL031736, AC010209, AC006257, Z93931, Z98884, Z95889, AL034402, AC004999, Z86064, AC004896, AL132796, AC000119, AL031431, AF099810, AP000014, AC002288, AF011889, AC005160, AC008170, AL021877, AC002390, AL049570, AC007677, AL049874, AC006600, AC002492, AL109760, AC005919, AP000158, AC004601, Z99497, Z54335, AC004506, AL049767, AC006354, AC007092, AC005684, AC005908, AC004385, AL031904, AL049710, AL035468, AC007225, AC008085, AL050334, Z84470, AL031737, AC004074, AC008127, Z84467, AC005230, AC002045, AL078583, AL021919, AC009514, AC004695, AP000208, AP000130, AC002067, AL135921, AC004070, AP000247, AL132800, AL109963, AL122023, AP000455, AP000069, AC007590, AC007446, AC002494, AC002386, AC005988, AL079352, AC005002, AC000392, AC004478, AC007347, AC005225, AC006568, AC005323, AL022397, AC004168, AP000566, AL133512, U73508, AL078474, AC007102, AF205588, Z98880, AL031732, AC006032, AC006536, AL117337, AF129107, AL033392, AC007680, AC004806, Z98304, AC008045, AP000704, AC000117, AL078612, AJ229042, AC002070, AC016831, AF051934, AP000008, AP000694, AC008069, AC005922, AL137617, AC006144, AC004106, Z97205, AC005863, AC002478, AC005155, AL079303, Z77249, AC011456, AB020868, AL035691, M74207, AC006210, AC002981, AC003960, AL031654, AC009405, AP000240, AL023581, AL035454, AP000339, AC008273, AP000108, AP000040, AF117829, AC004953, AP000282, AP000567, AL034411, AC008498, AC003686, AP000217, AF130342, AL049792, AL021578, AC005587, AC002299, AF064857, Z82189, AC004905, AP001043, AC005164, AC006961, AL121838, AL117353, AL109809, AC005685, AP000695, AC005186, AF064865, AF064862, AL049798, Z69906, AL035251, AC005252, and AC002368.
26	HNGMJ91	89	836365	AA766885, AL024508, AC003692, AC007216, AC006409, and AL078581.
27	HNGNB69	37	833405	AI539328 and AA701004.
28	HNGPM78	38	1008207	AI696793, AW162288, AL046409, AI963720, F13749, AW277171, AA130647, AA071334, AI821881, AI821918, AI358089, AW327624, AA533033, AI431434, AA563770, AI284640, AI076236, AA514020, AI635819, AI431303, AW303196, AA525508, AW301350, AA659627, AI537538, AI613280, AW274349, AI587583, AI587565, AW276827, AA410788, AW193265, AI350211, AW157005, AI457397, AI270117, AI367544, AI457597, AA584489, AA644320,

				AI017251, AI753365, AI133164, AI954525, AW271917, AW265385, AA657835, AI751162, AI745325, AL041706, AI028510, AI919265, AL037683, R83710, AA491814, AA828764, AI446601, AA992126, AA488746, AI375710, AI079423, AI434695, F27015, AA635304, AA088841, AA904137, AI619997, AI338426, AI268351, AI251104, AA574442, AA651639, AW410354, H57265, AI370878, AA829225, AW088202, AA228778, AA623002, AA610509, AW341903, AW007759, AI925321, AI598060, C05986, AI580652, AI915293, AI341664, AI205181, AA640986, AI291823, AI688846, AA482953, AI358501, AI267818, AI761471, AW029038, AW192065, AI336054, AW089589, AI246796, AW007662, H53168, AI801591, AI061334, AW193432, AI885488, AI085719, AA845293, AI003997, AW406755, AA652764, AA610491, AL046898, AI039809, AI090334, AA177061, AI341548, AA904274, AI278440, AI499938, AI277373, AI355587, AW162049, AI332615, AI312790, AI168185, AI929531, AW088846, AW021583, AA501614, AI471481, AI305766, AA865262, AW129001, AW062724, AL138455, AI445474, AA579184, AI570261, AI630283, AW192199, AI537955, AI564185, AI362552, AA657416, AI962050, AW439558, H79308, AL118991, AA613345, AW103030, AW270258, AW327868, AI829331, AC004660, AC007344, AC005726, AC007283, AL035448, AC005412, AL022578, AC005037, Z72521, Z82173, AL117330, AC005539, AL035398, AP000049, AL035413, Z73979, AL049869, AC005399, AP000311, AL022163, AL031005, AC004794, AC009516, AL109628, AC007364, AC005696, AL033538, AC004167, AC002059, AL022170, AL022313, AL049872, AC000026, AL031053, Z93244, AL021453, AP000151, X74984, AL035667, AL035587, AP000116, AC005242, AC005768, AC000353, AC005062, AC004922, AL021939, AC004021, AC004449, AL024509, AC007630, Z98950, AF015156, AL050332, L48038, U18391, AC007376, AC005877, AC004996, AC007262, AC007057, Z82244, AC005035, AC002117, AC007011, U18399, AF015151, AC002299, AC004231, AL117536, AC006080, Z99129, AC004832, AP000010, AL031255, AC005703, AC006390, AP000356, AP000558, AP000503, AC007590, Z97630, AC003964, AC009946, AL022322, AP000045, U57005, AL133245, AC006948, AC007038, U18396, AC004544, AC005498, AC005785, AC003101, D83989, AC007237, I51997, U85195, AC007792, U18394, AC004672, AC005839, AL034409, X53550, AL049776, AC004087, AC006512, AL049562, AL031777, M63796, AL034384, AE000658, AL035701,

				AL049867, U57009, AC007919, AL049759, AC006430, X54176, AP000557, AC007023, AC005823, AC000025, U18392, U18393, AC005229, AL022323, X55926, AL121653, AF134726, AL008735, U95740, AC005914, AC006530, AC004263, AL136504, X55925, AC006571, AC010202, AC005384, U57007, AL031685, AC007228, AC005154, AL024498, AC006241, AL117258, AL023279, AC004448, AC002074, U57008, AL033525, AC002492, AL118497, AC005046, U91326, AC004156, AL035089, AC003992, AL034555, AJ239027, U57004, AC008033, AC006236, AF015147, Z86062, AC004084, AC007842, Z99570, X76629, Y16790, AL022320, AC003683, AC004019, AL008715, X54180, AC005829, AP000460, AL031230, AF015157, AC002104, AC004655, AC002531, U18390, AC007487, AC005049, AC003667, AC000092, AC004887, AL035405, AC007688, AC005899, X74558, AL022165, AP000552, AL034417, AC005900, AC005618, X55931, AL008631, AC006468, AJ003147, AC004049, AC007993, AL023575, AC004921, AL049569, AC006271, AL049709, AF077058, AC006064, AC004080, AC006292, AC005181, AC003080, U57006, AC003692, AC002540, AC007350, AC005901, Z98304, AL049610, AB022785, AC008055, S75201, AC004491, AL078614, AL132708, AC004967, AC005280, AL049874, AP000502, U18387, AP000692, AC008119, AC002480, AC005324, AF015149, AL035450, L34079, AC004706, and AC006356.
28	HNGPM78	90	895289	AC004660.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

Vector Used to Construct Library	Corresponding Deposited Plasmid
Lambda Zap	pBluescript (pBS)
Uni-Zap XR	pBluescript (pBS)
Zap Express	pBK
lafmid BA	plafmid BA
pSport1	pSport1
pCMVSport 2.0	pCMVSport 2.0
pCMVSport 3.0	pCMVSport 3.0
pCR®2.1	pCR®2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey

Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5′ NT and the 3′ NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5′ or 3′ non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5′ and 3′ "RACE" protocols which are well known in the art. For instance, a method similar to 5′ RACE is available for generating the missing 5′ end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5′ ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5′ portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5′ phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5′ ends of messenger RNAs. This reaction leaves a 5′ phosphate group at the 5′ end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5′ end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5′ end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue Distribution of Polypeptide

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P^{32} using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds,95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptid

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with

high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfuidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive

Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral

sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of ^{35}S-methionine and 5 uCi ^{35}S-cysteine (available from Amersham) are added.

The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of

interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector

are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five µg of the expression plasmid pC6 a pC4 is cotransfected with 0.5 ug of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 uM, 2 uM, 5 uM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 uM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused

protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:
GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGC
CCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGT
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
ACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG

GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

Example 10: Production of an Antibody from a Polypeptide

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Example 11: Production Of Secreted Protein For High-Throughput Screening Assays

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described herein.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x 10^5 cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl2 (anhyd); 0.00130

mg/L $CuSO_4$-$5H_2O$; 0.050 mg/L of $Fe(NO_3)_3$-$9H_2O$; 0.417 mg/L of $FeSO_4$-$7H_2O$; 311.80 mg/L of Kcl; 28.64 mg/L of $MgCl_2$; 48.84 mg/L of $MgSO_4$; 6995.50 mg/L of NaCl; 2400.0 mg/L of $NaHCO_3$; 62.50 mg/L of NaH_2PO_4-H_20; 71.02 mg/L of Na_2HPO4; .4320 mg/L of $ZnSO_4$-$7H_2O$; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H_20; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H_20; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H_20; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalainine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tryrosine-2Na-$2H_20$; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B_{12}; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person

B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 12: Construction of GAS Reporter Construct

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

Ligand	JAKs tyk2	Jak1	Jak2	Jak3	STATS	GAS(elements) or ISRE
IFN family						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
Il-10	+	?	?	-	1,3	
gp130 family						
IL-6 (Pleiotrophic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
Il-11(Pleiotrophic)	?	+	?	?	1,3	
OnM(Pleiotrophic)	?	+	+	?	1,3	
LIF(Pleiotrophic)	?	+	+	?	1,3	
CNTF(Pleiotrophic)	-/+	+	+	?	1,3	
G-CSF(Pleiotrophic)	?	+	?	?	1,3	
IL-12(Pleiotrophic)	+	-	+	+	1,3	
g-C family						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
gp140 family						
IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
Growth hormone family						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
Receptor Tyrosine Kinases						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCC GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':<u>CTCGAG</u>ATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCGAAA TGATTTCCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCG CCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCT CCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCC TCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCT AGGCTTTTGCAAA<u>AAGCTT</u>:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, Il-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 13: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml genticin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1 x 10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at –20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 14: High-Throughput Screening Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2x10e^7 U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM $Na_2HPO_4.7H_2O$, 1 mM $MgCl_2$, and 675 uM $CaCl_2$. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting $1x10^8$ cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of $5x10^5$ cells/ml. Plate 200 ul cells per well in the 96-well plate (or $1x10^5$ cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count

the cell number and add more low serum medium to reach final cell density as $5x10^5$ cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to $1x10^5$ cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

Example 16: High-Throughput Screening Assay for T-cell Activity

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence

complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCC ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA GCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described

in Example 13. As a positive control, exogenous TNF alpha (0.1,1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

Example 17: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5

25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO_2 incubator for

20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4 , 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO_2 incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10^6 cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10^6 cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4 . The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca^{++} concentration.

<u>Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity</u>

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is

unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in

Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 and a cocktail of protease inhibitors (# 1836170) obtained from Boeheringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg_{2+} (5mM ATP/50mM $MgCl_2$), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM $MgCl_2$, 5 mM $MnCl_2$, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20

min. This allows the streptavadin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phospotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and

cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United

States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in

Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 23: Formulation

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials

(for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized

formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate

administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892),TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In

another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin,

erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-

acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compostions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered

with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Gorwth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but

are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 24: Method of Treating Decreased Levels of the Polypeptide

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

Example 25: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of

decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

Example 26: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is

maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935

(1989); and Zijlstra et al., *Nature,* 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM $Na_2 HPO_4$, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately $3X10^6$ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 μg/ml. 0.5 ml of the cell suspension (containing approximately $1.5.X10^6$ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 28: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or

precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg

body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper

dosages and other treatment parameters in humans and other animals using naked DNA.

Example 29: Transgenic Animals.

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding

strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 30: Knock-Out Animals.

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas &

Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For

example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 31: Production of an Antibody

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide(s) of the invention, or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 μg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line

(SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding polypeptide(s) of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide(s) of the invention protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide(s) of the invention protein-specific antibody and are used to immunize an animal to induce formation of further polypeptide(s) of the invention protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed polypeptide(s) of the invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the

invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to innoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to

stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 μg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can,

in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

<u>In Vitro Assay-</u> Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed Staphylococcus aureus Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

<u>In Vivo Assay-</u> BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice

receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 33: T Cell Proliferation Assay

Proliferation assay for Resting PBLs.

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^{3}H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 microliters per well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 C (1 microgram/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5×10^4/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of TNF Delta and/or TNF Epsilon protein (total volume 200 microliters). Relevant protein buffer and medium alone are controls.

After 48 hr. culture at 37 C, plates are spun for 2 min. at 1000 rpm and 100 microliters of supernatant is removed and stored -20 C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 microliters of medium containing 0.5 microcuries of ^{3}H-thymidine and cultured at 37 C for 18-24 hr. Wells are harvested and incorporation of ^{3}H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TNF Delta and/or TNF Epsilon proteins.

Alternatively, a proliferation assay on resting PBL (peripheral blood lymphocytes) is measured by the up-take of ^{3}H-thymidine. The assay is performed as follows. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non-adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2 x10^4 cells/well in a final volume of 200 microliters. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2 (*), IFNγ, TNFα, IL-10 and TR2. In addition to the control supernatants, recombinant human IL-2 (R & D Systems, Minneapolois, MN) at a final concentration of 100ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^{3}H-thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^{3}H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

(*) The amount of the control cytokines IL-2, IFNγ, TNFα and IL-10 produced in each transfection varies between 300pg to 5ng/ml.

Costimulation assay.

A costimulation assay on resting PBL (peripheral blood lymphocytes) is performed in the presence of immobilized antibodies to CD3 and CD28. The use of antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through stimulation of the T cell receptor by an antigen. Cross-linking of the TCR (first signal) in the absence of a costimulatory signal (second signal) causes very low induction of proliferation and will eventually result in a state of "anergy", which is characterized by the absence of growth and inability to produce cytokines. The addition of a costimulatory signal such as an antibody to CD28, which mimics the action of the costimulatory molecule. B7-1 expressed on activated APCs, results in enhancement of T cell responses including cell survival and production of IL-2. Therefore this type of assay allows to detect both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

The assay is performed as follows. Ninety-six well plates are coated with 100ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 100ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2 x10^4 cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only (negative control), IL-2, IFNγ, TNFα, IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is

pulsed with 1uCi of ^{3}H-thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^{3}H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

Costimulation assay: IFN γ and IL-2 ELISA

The assay is performed as follows. Twenty-four well plates are coated with either 300ng/ml or 600ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 500ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the costimulation assay. The assay is performed in the pre-coated twenty-four well plate using 1 x 10^5 cells/well in a final volume of 900ul. The supernatants (293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 300ul are added to 600ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only(negative control), IL-2, IFNγ, IL-12 and IL-18. In addition to the control supernatants recombinant human IL-2 (all cytokines were purchased from R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml, IL-12 at a final concentration of 1ng/ml and IL-18 at a final concentration of 50ng/ml are also used. Controls and unknown samples are tested in duplicate. Supernatant samples (250ul) are collected 2 days and 5 days after the beginning of the assay. ELISAs to test for IFNγ and IL-2 secretion are performed using kits purchased from R & D Systems, (Minneapolis, MN). Results are expressed as an average of duplicate samples plus or minus standard error.

Proliferation assay for preactivated-resting T cells.

A proliferation assay on preactivated-resting T cells is performed on cells that are previously activated with the lectin phytohemagglutinin (PHA). Lectins are polymeric plant proteins that can bind to residues on T cell surface glycoproteins including the TCR and act as polyclonal activators. PBLs treated with PHA and then cultured in the presence of low doses of IL-2 resemble effector T cells. These cells are generally more sensitive to further activation induced by growth factors such as IL-2. This is due to the expression of high affinity IL-2 receptors that allows this population to respond to amounts of IL-2 that are 100 fold lower than what would have an effect on a naïve T cell. Therefore the use of this type of cells might enable to detect the effect of very low doses of an unknown growth factor, that would not be sufficient to induce proliferation on resting (naïve) T cells.

The assay is performed as follows. PBMC are isolated by F/H gradient centrifugation from human peripheral blood, and are cultured in10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD) in the presence of 2ug/ml PHA (Sigma, Saint Louis, MO) for three days. The cells are then washed in PBS and cultured in10% FCS/RPMI in the presence of 5ng/ml of human recombinant IL-2 (R & D Systems, Minneapolis, MN) for 3 days. The cells are washed and rested in starvation medium (1%FCS/RPMI) for16 hours prior to the beginning of the proliferation assay. An aliquot of the cells is analyzed by FACS to determine the percentage of T cells (CD3 positive cells) present; this usually ranges between 93-97% depending on the donor. The assay is performed in a 96 well plate using 2 $\times 10^4$ cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of in10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2, IFNγ, TNFα, IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of ^{3}H-thymidine(Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of ^{3}H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

The studies described in this example test activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF-α, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCγRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Thl helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6/ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell

cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e..g, R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

<u>Monocyte activation and/or increased survival.</u> Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

<u>Monocyte Survival Assay</u>. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results

from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2 x 10^6/ml in PBS containing PI at a final concentration of 5 μg/ml, and then incubaed at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5x10^5 cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e..g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at 2-1x10^5 cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 μl 1N NaOH per well. The absorbance is read at 610

nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 35: Biological Effects of Polypeptides of the Invention

Astrocyte and Neuronal Assays·

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA 83*:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays·

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE_2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1α for 24 hours. The supernatants are collected and assayed for PGE_2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP^+) and released.

Subsequently, MPP^+ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP^+ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotidamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined in vitro in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm^2 on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at 2-5x10^4 cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnique, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, $VEGF_{165}$ or a

polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol. 30A*:512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 39: Stimulation of Endothelial Migration

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5×10^5 cells suspended in 50 ul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO_2 to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 40: Stimulation of Nitric Oxide Pr duction by End thelial Cells

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be

assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:

$$2\ KNO_2 + 2\ KI + 2\ H_2SO_4\ 6\ 2\ NO + I_2 + 2\ H_2O + 2\ K_2SO_4$$

The standard calibration curve is obtained by adding graded concentrations of KNO_2 (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H_2SO_4. The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per $1x10^6$ endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm. 217*:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 41: Effect of Polypepides of the Inventi n on C rd Formation in Angiogenesis

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 ml/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 mg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese qual (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old qual embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse

In vivo angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the

Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 44: Rescue of Ischemia in Rabbit Lower Limb Model

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol 147*:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita*et al. Am J. Pathol 147*:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al. Hum Gene Ther. 4*:749-758 (1993); Leclerc *et al. J. Clin. Invest. 90*: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow

during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number m the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

Example 45: Effect of Polypeptides of the Invention on Vasodilation

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as $p<0.05$ vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 46: Rat Ischemic Skin Flap Model

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

a) Ischemic skin

b) Ischemic skin wounds

c) Normal wounds

The experimental protocol includes:

a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 47: Peripheral Arterial Disease Model

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

c) The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 48: Ischemic Myocardial Disease Model

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 49: Rat Corneal Wound Healing Model

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.

c) Making a pocket (its base is 1-1.5 mm form the edge of the eye).

d) Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

e) Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

A. Diabetic db+/db+ Mouse Model.

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al., J. Surg. Res. 52*:389 (1992); Greenhalgh, D.G. *et al., Am. J. Pathol. 136*:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al. Proc. Natl. Acad. Sci. USA 77*:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al., J. Immunol. 120*:1375 (1978); Debray-Sachs, M. *et al., Clin. Exp. Immunol. 51(1)*:1-7 (1983); Leiter *et al., Am. J. of Pathol. 114*:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al., Exp. Neurol. 83(2)*:221-232 (1984); Robertson *et al., Diabetes 29(1)*:60-67 (1980); Giacomelli *et al., Lab Invest. 40(4)*:460-473 (1979); Coleman, D.L., *Diabetes 31 (Suppl)*:1-6 (1982)). These

homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al., J. Immunol. 120*:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al., Am. J. of Pathol. 136*:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med. 172*:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm^2, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al., Am. J. Pathol. 136:*1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary

antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

B. Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl*et al., J. Immunol. 115*: 476-481 (1975); Werb *et al., J. Exp. Med. 147*:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al., An. Intern. Med. 37*:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al., Growth Factors. 5:* 295-304 (1991); Haynes *et al., J. Clin. Invest. 61:* 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes et al., *J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In:* Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al., Growth Factors. 5:* 295-304 (1991); Haynes *et al., J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In*: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al., Proc. Natl. Acad. Sci. USA 86*: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad*

libitum. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8).

The wound area on day 1 is $64mm^2$, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 51: Lymphadema Animal Model

or The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing.

Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosis and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca2+ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and

extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO_2. HUVECs are seeded in 96-well plates at concentrations of 1 x 10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution

of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone

has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5 x 10^5 cells/ml. During this time, 100 μl of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μl of prepared cytokines, 50 μl SID (supernatants at 1:2 dilution = 50 μl) and 20 μl of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 μl. The plates are then placed in a 37°C/5% CO_2 incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μCi/well of [3H] Thymidine is added in a 10 μl volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 μl Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined

via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECR)

The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5.\beta_1$ and $\alpha_4.\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating

stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of 0.2 µg/ cm^2. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Gene products are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2, 7% O_2, and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular gene product is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses

of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 μl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μg/ml hEGF, 5mg/ml insulin, 1μg/ml hFGF, 50mg/ml gentamycin, 50 μg/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for

AoSMC contains SM basal media, 50mg/ml gentamycin, 50μg/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO_2 until day 5.

Transfer 60μl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100 μl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10μl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 μl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 μl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 μl/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100 μl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or

smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vasculargenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 µl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 µl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 µl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution, refered to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of

the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 57: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The

plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml,

Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2 x 10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2 x 10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 μC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Additionally, the contents of U.S. Provisional Applications Serial Nos. 60/163,581 and 60/215,133 are all hereby incorporated by reference in their entirety.

Applicant's or agent's file reference number	PS708PCT	International application No. PCT/US 00/30045

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 67, line N/A.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
13 October 1999	PTA-847

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).
Continued on the Attached Pages 2 & 3

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

ATCC Deposit No. PTA-847
Page N . 2

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

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Page No. 3

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;

(e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:X;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:X;

(h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;

(i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(g) a variant of SEQ ID NO:Y;

(h) an allelic variant of SEQ ID NO:Y; or

(i) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

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<212> DNA
<213> Homo sapiens

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<212> DNA
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<211> 1769
<212> DNA
<213> Homo sapiens

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<212> DNA
<213> Homo sapiens

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<211> 2340
<212> DNA
<213> Homo sapiens

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<210> 34
<211> 2128
<212> DNA
<213> Homo sapiens

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<210> 35
<211> 2034
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1911)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1956)
<223> n equals a,t,g, or c

<400> 35

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gattgcagaa ctgtccttcg ctgcagctgt aaaatcgcgg caaggcagga ttcatcctgg      360
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agctcttttc tttataaaaa aaaaaaraaa aaactcgagg gggggcccgg nacccaattc     1920
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<210> 36
<211> 638
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (573)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (605)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (613)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (624)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (630)
<223> n equals a,t,g, or c

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<210> 37
<211> 715
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (354)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (618)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (637)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (646)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (694)
<223> n equals a,t,g, or c

<400> 37
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aagtaactaa agatgcataa ataactataa gccttccatg tggtaccaaa gagaagtgga 180
atgggagtta gggaaagcag aagctactga gggaagtgga gctcacagtg atcttcaagg 240
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<210> 38
<211> 1747
<212> DNA
<213> Homo sapiens

<400> 38
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<210> 39
<211> 2243
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (3)
<223> n equals a,t,g, or c

<400> 39
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<210> 40
<211> 667
<212> DNA
<213> Homo sapiens

<400> 40
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<210> 41
<211> 788
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (717)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (737)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (764)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (777)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (779)
<223> n equals a,t,g, or c

<400> 41
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<210> 42
<211> 793
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (7)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (14)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (76)
<223> n equals a,t,g, or c

<400> 42
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<210> 43
<211> 1005
<212> DNA
<213> Homo sapiens

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<223> n equals a,t,g, or c

<220>
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<223> n equals a,t,g, or c

<220>
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<223> n equals a,t,g, or c

<220>
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<222> (977)
<223> n equals a,t,g, or c

<400> 43
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<211> 675
<212> DNA
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<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

<220>
<221> SITE

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<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (660)
<223> n equals a,t,g, or c

<400> 44
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gtgtggtggg cactgaggct gcagtgtgcc atgatcgtgc ctctgcactc cagcctgggc     180
gacagagcaa gaccctgtct caaagaacaa aagtgacaaa cgtagcacta aatagaatac     240
aacaagaaca cttatttaca gcatgagcgc taagggagca ggcagtgttg cctcattcac     300
actcagctgg gaaagtgggt gtcaggtgac ttgaatttgt tgccgaaagc agggcagata     360
tttatttggg gattacmrat aaatcttagc aagcgagatt ctcagataca gagtctacga     420
acaatcaggg ctgcccgtag ctgctgccty ccacaggagg tgcccctggg cagtccccar     480
gccctgacct gcagtttctg tgaaaacacc atcagccctg ggggtctctt tcagtgtcca     540
gccattcaga gaccaaatta cgggcctgct accttctaga cttgngacct gggcaaagtc     600
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ttctggggat ccaag                                                     675
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<210> 45
<211> 2086
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (10)
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<220>
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<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2075)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2079)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2083)
<223> n equals a,t,g, or c

<400> 45
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cggagagccc tcggcatcgg cttccagtgg ctctctttgg ccactctggt gctcatctgc 240
gccgggcaag gggacgcag ggaggatggg ggtccagcct gctacggcgg atttgacctg 300
tacttcattt tggacaaatc aggaagtgtg ctgcaccact ggaatgaaat ctattacttt 360
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agggccagtg agcagattta ttatgaaaac agacaagggt acaggacagc cagcgtcatc 600
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agaagtaaca tccacccatt cagaagccag ggttacatat ctaaacactc aatgagacca 2040
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<210> 46
<211> 722
<212> DNA
<213> Homo sapiens

<220>
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<222> (501)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (607)
<223> n equals a,t,g, or c

<220>
<221> SITE
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<220>
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<222> (670)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (693)
<223> n equals a,t,g, or c

<400> 46
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tt 722

<210> 47
<211> 4303
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (20)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (24)
<223> n equals a,t,g, or c

<400> 47
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tgtgctgccc ctcacgctcc tccatcctca ccggcaagta cgtccacaac cacaacacct 540
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<210> 48
<211> 1146
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1)
<223> n equals a,t,g, or c

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<223> n equals a,t,g, or c

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<222> (853)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (870)
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cagatg 1146

<210> 49
<211> 158
<212> DNA
<213> Homo sapiens

<400> 49
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cgacctcagg tgatccgccc gcctcagtct cccaaagtgc tgggattaag gcatgagcca 120
ccgcgcccgg tctttttttt tttttttttt tttttca 158

<210> 50
<211> 768
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (7)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (707)
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<220>
<221> SITE
<222> (726)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (743)
<223> n equals a,t,g, or c

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aatttctttt ataatatgct ttccgcagtc ctaactatgc tgcgttttat aatagctttt 180
tcccttctgt tctgttcatg tagcacagat aagcattgca cttggtacca tgctttacct 240
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tttaaaatac tgatattttt atttgktaat aatactttgc cctcagaaag attctgatac 600
cctgccttga caacatgaaa cttgaggctg ctttggttca tgaatccagg tgttcccccg 660

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<210> 51
<211> 1392
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (3)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (4)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (6)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (13)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (18)
<223> n equals a,t,g, or c

<400> 51

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aannanaact acntccgntt ttaggtttag gggcctggc cttgctgctg ttttgattta        60
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taggtcattt ttgtctgtgg catttttact gtttgtgaaa gtatgaaaca gatttgttaa      180
ctgaactctt aattatgttt ttaaaatgtt tgttatattt cttttctttt ttcttttata      240
ttacgtgaag tgatgaaatt tagaatgacc tctaacactc ctgtaattgt cttttaaaat      300
actgatattt ttatttgtta ataatacttt gccctcagaa agattctgat accctgcctt      360
gacaacatga aacttgaggc tgctttggtt catgaatcca ggtgttcccc cggcagtcgg      420
cttcttcagt cgctccctgg aggcaggtgg gcactgcaga ggatcactgg aatccagatc      480
gagcgcagtt catgcacaag gccccgttga tttaaaatat tggatcttgc tctgttaggg      540
tgtctaatcc ctttacacaa gattgaagcc accaaactga gaccttgata cctttttta       600
actgcatctg aaattatgtt aagagtcttt aacccatttg cattatctgc agaagagaaa      660
ctcatgtcat gtttattacc tatatggttg ttttaattac atttgaataa ttatattttt      720
ccaaccactg attacttttc aggaatttaa ttatttccag ataaatttct ttattttata      780
ttgtacatga aaagttttaa agatatgttt aagaccaaga ctattaaaat gatttttaaa      840
gttgttggag acgccaatag caatatctag gaaatttgca ttgagaccat tgtattttcc      900
actagcagtg aaaatgattt ttcacaacta acttgtaaat atattttaat cattacttct      960
ttttttctag tccattttta tttggacatc aaccacagac aatttaaatt ttatagatgc     1020
actaagaatt cactgcagca gcaggttaca tagcaaaaat gcaaaggtga acaggaagta     1080
aatttctggc ttttctgctg taaatagtga aggaaaatta ctaaaatcaa gtaaaactaa     1140
tgcatattat ttgattgaca ataaaatatt taccatcaca tgctgcagct gtttttaag      1200
gaacatgatg tcattcattc atacagtaat catgctgcag aaatttgcag tctgcacctt     1260
atggatcaca attaccttta gttgtttttt ttgtaataat tgtagccaag taaatctcca     1320
ataaagttat cgtctgttca aaaaaaaaaa aaaaaaaaaa aagctcgagg gggggaccgg     1380
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tacccgggct ga 1392

<210> 52
<211> 1992
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (7)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (31)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (42)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (45)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1977)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1992)
<223> n equals a,t,g, or c

<400> 52
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tggcggccgc tctagaacta gtggatcccc cggctgcag gaattcggca cgaggccgag 120
gctgcgcgcc ggctgctcct ccccaccccc agcctttgcc ctgaaggggg ctggatgggc 180
aaggcggccg cgatggctcg agctcgggcg gtggcggcgg tggccggagg cggcggtgcc 240
tcctcctcct cgccccggcg ccggcggtga tccgagcgag cggccgcggc ccccgatgag 300
actgctggcg ggctggctgt gcctgagcct ggcgtccgtg tggctggcgc ggaggatgtg 360
gacgctgcgg agcccgctca cccgctccct gtacgtgaac atgactagcg gcccgggtgg 420
gccggcggcg gccgcgggcg gcaggaagga gaaccaccag tggtatgtgt gcaacagaga 480
gaaattatgc gaatcactcc aggctgtctt tgttcagagt taccttgatc aaggaacaca 540
gatcttctta aacaacagca ttgagaaatc gggctggcta tttatccaat tatatcattc 600
ttttgtgtca tctgttttta gcctgtttat gtctagaaca tctatcaatg ggttgctagg 660
aagaggctca atgtttgtgt tttcaccaga tcagtttcag agactgctta aaattaatcc 720
agactggaaa acccacagac ttcttgattt aggtgctgga gatggagaag tcacaaaaat 780
catgagccct cattttgaag aaatctatgc cactgagctt tctgaaacta tgatatggca 840
gcttcagaaa aagaaataca gagtccttgg tataaatgaa tggcagaata cggggttcca 900
gtatgatgtc atcagctgcc tgaacttgct ggaccgctgt gatcagcccc tgactttgtt 960
aaaagatatc agaagtgtct tggagccaac tagaggcagg gtcatccttg cccttgtcct 1020
cccctttcat ccctatgtgg aaaacgtagg tggcaagtgg gagaaaccat cagaaatttt 1080
ggaaatcaaa ggacagaact gggaagaaca agtgaatagt ctgcctgaag ttttcagaaa 1140

agctggtttt gttatcgaag ctttcaccag actaccatac ctgtgtgaag gcgacatgta 1200
taatgactac tacgttctgg atgacgctgt ctttgttctc aaaccagtat aaacacgtgg 1260
aggtcgaagt cttcagagtc cgcaccctcc gggatgtgcc cttggaagag ggtctgtgtt 1320
cacaattacg tgaagggagg acccttgggg accgccattc taaatatcat gtaggaattt 1380
aaaaagccaa aatactaatt atttctttgt agtgtgtaaa ggaatgtttt taaaagacaa 1440
aaacccaact ctttgtggat ttttatcaac tctttactca gagccactct ccaatgcagg 1500
tcacactcca attatgatgg aagatatttt ttatacttaa ttgcagtagg gactcattcc 1560
cagacaaagc aatagtcacg acttcatgga accaatcaat ggattgtttt ttgaagactg 1620
gcaataaagc tgtccattca attccaaata ctggttttaa ggtatagcca ctgatattct 1680
ttcatgttta gaaattcttt ctgttattat tcaagaaaat gtttttaatc atgctaataa 1740
actttttttgg agatgacttt ggcatcatgt ttgaattcat ataaagctcc cctagcattt 1800
tttattggtt tggcttcagg agtacccaaa tagtagcatt atgagaatga cgcagacaat 1860
ttgaataggg gggaaggaag gcttcagact tgggggaagg ggagattatt gcaaattgca 1920
gtgaacactg agtcagtaaa aaaaaaaaac tcggaggggg gmmcsgwacc cctttgnccc 1980
tttgggggc cn 1992

<210> 53
<211> 791
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (596)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (607)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (651)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (680)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (765)
<223> n equals a,t,g, or c

<400> 53
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ttgttaagag aagggtggga atgaatggga tctcatcatg gatttccaat aggatacaag 180
cgccctacca gccactatca tcccacatca acctagaacg aattctytct cagcttgccc 240
tgscccctgs cctgcccaga gtctgtccaa gcagactcta ccacagacag gagaaagctg 300
aggcagcaga acaagagacg ggagaggcca ggtttgggtg aggggcccta cccccaccag 360
ggtcagcttg ggtgtgctg gagccagagt cctgtgccag gaggcagaag gggcaccttg 420
aaagaagctc cccacaccct tcaaccctgg cacagcccag ccagccaccc aggtaatgca 480
cttaaagtgc tcagcaccca gtcagcgttg accactcttc tcgttatata ttaaatacaa 540
caaacatctt tcaagaacaa aaagcaagca tgtttggtcc attcatgaat ccttgnacac 600

tgattgntcg ggttctttca gttcccatcc acttagaaat aacaaacatg nttcattttg 660
agcctatcct acagggccgn aaatccggag cctgcaagtt tggtctcttg gcctggggcc 720
aagggtgcag atggttgaag cccttggatc cctgagtggc tgcgnctccc aagtctgggc 780
tgactcatgg g 791

<210> 54
<211> 1265
<212> DNA
<213> Homo sapiens

<400> 54
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caggccgtac cctttatttc ctcctccgtt tcatttggct cccagtggct gctctgcggt 120
tttgatttcc ctccagctat ggcactgaat tcatttatgg aaagagaccc accacctaat 180
tggcggatga gcttgggctt ctgggtgtgg cttccttctt gctgtcataa gatgctggtg 240
gtgacctgca catttggcca ctacctcccg ctggaatcca gccaccatct gtragatggg 300
gcatgacgcg gtccagtgga gggtgcgtgc tggagtctct cccgtctcca ccacctttgt 360
tacagatgtt ctttccgaac gtaggtctct gccgtctctt acttgtttaa aacgcccaga 420
acccgagtca gccttggcag tgagcttacg gcccgcacca ggaggggcca gcctgctgcc 480
aagatgggga cggttccctg ggccccgcgg tctgcggtgc cgcctcccgc ttcaccgcac 540
ggtgctctct ttcccccacc ccccctcaga agcgccggca tattccagag gagtcaacaa 600
acaaatggaa gcagagggtt aagacagcca tggcgggggt gaaattggta cttttctatt 660
ttgcgggcag cgcgcgtctt gactgtttgc aggcaatcaa agtcgattgt gtttttcctc 720
aacaaactgt tgtatatttt tttttkaaca ttcacatccg tgtttctctg tcttccttcg 780
gcacccagtg agcccagcat gttgcgatgg cactgcatgg ctatggtggg tctgcggtgg 840
cggrgacggr ggtggggctc tggcttgact tggagctcgt gaaacgtgct tgtcacaact 900
gcctgcaagt gtttcttggt ctcctcgtta acatgcatgc caccccaccc agaacgagtg 960
gcctccggtg tcatgccccgt ctgcattctg tgacctaata catgcatttt cccatggaaa 1020
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gaccaagcca accctgggtt ctgagagcct tcaggacatg tgtcaccgca gacgacgttt 1140
taacttgtag tgctagctgc ttgtttcaac ttgctccaca aaggaaaaat aactattatg 1200
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ctttt 1265

<210> 55
<211> 595
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (15)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (16)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (374)
<223> n equals a,t,g, or c

<220>
<221> SITE

<222> (450)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (468)
<223> n equals a,t,g, or c

<400> 55
gcggccgccc ttttnntttt tttttttttg agacagagtc ttgctctgtc acccaggctg 60
gagtgcagtg gtgtgatctt ggctccccgc aacctctgcc tcccgggttc aagcaattgt 120
cctgcctcag cctcctgagt agctgggact acaggcatgc accaccacac cagctaattt 180
ttgcattttt agtaagagac ggggtttcac catattggcc aggctggtct cgaactcctg 240
acctcaagtg atctgcccgc ctcggcctcc caaagtgctg ggattacagt cgtgaaccac 300
cacgcccggc tgaagccaat cacttccttg cggtttcccc tgacttcgtc tggccttaac 360
cttctggaac ttancatgaa gatgatggag accaagttag tgaggtaggc tgggaaccga 420
tctctccatg tcagcttcac tttgtcagtn tacaacacaa aacaatgncg gtcaactgtc 480
tggaaccatc acgatgagac atctctggtg tgtcagcgtg ggtgcatgtt gccgtgcgtg 540
catggggccc agggacaact tggaacaacc aggaaatact cagaaaaaac ctcaa 595

<210> 56
<211> 1013
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (317)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (728)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (749)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1013)
<223> n equals a,t,g, or c

<400> 56
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ccagggtggg ggtagttttg tgggaaagca gaactgaagt tagcttaagc ataaaaacaa 120
agaaaaatct tcgcttttca tgtatgtgga atccaagaat aaccataggc tctaccagac 180
cagragggta aggatggaca ctaaaatgaa acaaatacca aggtattcct tctgctgcag 240
cctggagacc accgagagtc gagctggggc acacacacac ctggccggga cccgcaggga 300
caaggcgggc cgtggcntcc tccaccaagt ctctctagac aattcagggc ctgctttccc 360
cagctccatg catggctgga ctggtgattc cagggtgcag aagggattca tattcccaga 420
acgctttaag tgtacacctg caggataaag agataccggt tacattatta aatgattcta 480
gggattcact gggggatatt tttgttgctt ttactttcat ggttagagct acaaagaaca 540
gtgatttttt ttttttctcc cttccccatt cagaaacatt atacattggg ccatttttct 600
ttctcccaaa gaagattcat ggatagtcag actgaactgt gtgcaacagg aaaagtcaaa 660

agggaaaagg yrgctgatga ggttacatgg ttacatgktc tacatcatgc akagtagctt 720
gaaatctnag tctggagaaa actggatcna agattctagc ccactggagt tgcaaggaat 780
gagaggcaaa aattctaaag atttgggtta tattttcaac ttgggggaca gagagaaatg 840
gagagcagga attacagttc caacaaacat catgatagkc tggkagtcaa gacagagatt 900
aagtaaaaca ggttttactg kttagctgag ttcaggtaat acmaaatgwc ataaaacgkt 960
agtcctttga gactgacatg attaatgatc artgtggtgg gaaatgattg ggn 1013

<210> 57
<211> 701
<212> DNA
<213> Homo sapiens

<400> 57
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gctttaataa gtgttgacaa cactgttttg caaaatgtaa agrtactata caaattctta 120
atacaaaaag aataaattaa aagcagattt cttttttttaa ttctgcaact ttgtctacaa 180
cgtacatctt tttcattgat tacagttgaa cagaatccag taaaatcatt ttacatgctc 240
tacagtcagt ttcaggagca acctaatctt ttttccccca ttattaaact agagtccatt 300
ttacacaact tgtaataaac tattgacatt aatgtatatg taaaacttta cacctagtta 360
actaagcagt aactggtcat ctgatagcac ctggatgggg tttgctatat ttagaactaa 420
actaatactg aatgaaaaca aattggaatt ttaacagttt caacactcac ctgcatataa 480
taaaataaaa aatcaacctg gctcacaaca tagtctgatg tatgtgtttt caccagtctt 540
ctggctgaaa caggcaattt cttttacgca tccgaagaat ccartagggg ctttatatca 600
gaccaaggat tcgtatttca ccttattttg ttttaaagaa atagggataa agagtttgca 660
agtgcttgtg tacctcggcc gcgaccacgc taagccgaat t 701

<210> 58
<211> 814
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (3)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (10)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (556)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (813)
<223> n equals a,t,g, or c

<400> 58
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ttagagctac aaagaacagt gatttttttt ttttctccct tccccattca gaaacattat     120
acattgggcc atttttcttt ctcccaaaga agattcatgg atagtcagac tgaactgtgt     180
gcaacaggaa aagtcaaaag ggaaaaggca gctgatgagg ttacatggtt acatgttcta     240
catcatgcag agtagcttga aatctagtct ggagaaaact ggatcaagat tctagcccac     300
tggagttgca aggaatgaga ggcaaaaatt ctaaagattt gggttatatt ttcaacttgg     360
gggacagaga gaaatggaga gcaggaatta cagttccaac aaacatcatg atagtctggt     420
agtcaagaca gagattaagt aaaacaggtt ttactgttta gctgagttca gttaatacaa     480
aatgtacata aaacgttagt cctttgagac tgacatgatt aatgatcagt gtggtgggaa     540
atgatgtagt tattgnacac aagcacttgc aaactcttta tccctatttc tttaaaacaa     600
aataaggtga aatacgaagt ccttggtctg atataaagcc cctattggat tcttcggatg     660
cgtaaaagaa attgcctgkt tcagccagaa gactggtgaa aacacatacm tyagactatg     720
ttgtgagcca ggttgatttt twattttatt atatgccagg tgagtggttg aaactggtaa     780
aaattcccat ttggtttcat tcagtattag gtnc                                 814
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<210> 59
<211> 1308
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1146)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1161)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1169)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1183)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1239)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1245)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1249)
<223> n equals a,t,g, or c

<220>

<221> SITE
<222> (1302)
<223> n equals a,t,g, or c

<400> 59
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tcaaggatct gctgtagcat caagctttgg caacatgatg agtaaagaaa agcgagattc 120
catcagcaag gaagacctcg cccgggccac attggtcacc atcaccaaca acattggctc 180
cattgctcgg atgtgtgcgt tgaatgagaa catagacaga gttgtgtttg ttggaaattt 240
tctcagaatc aatatggtct ccatgaagct gctggcatat gccatggatt tttggtccaa 300
aggacaactg aaagctctgt tttggaaca tgagggttat tttggagccg ttggggcact 360
gttggaactg ttcaaaatga ctgatgayaa gtagagacga gcagtggagg aaacagcctc 420
ccaaaaggac agagaactaa aaaattgctg ctggagaagg tgaaagtcgc tttgggacgg 480
aagccaagcc attatggcag atgaacctgc tggatttgta aataatttaa aatccttcca 540
gatgatcttt tactcttagg ttttgagcta atgattcaaa acgggggaat ataaaaggtt 600
ttttttctgt atactgtatt tttttaaaaa aatggtgcag cgtggccaaa cctaccaatt 660
gtatgcatta actttgaaaa gttgtttgat gtttaagaag gacctgatat gtaagcgctg 720
gtcatttttc ttctggggtt tactgatcag tgtggtgatt ttaacttcat ttagtaatta 780
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tcaaatgaaa ctacaaatct ggcatgtttt actgtgaaca cttttgwtat ttgktttgta 900
cccttttttg tcttgktttt ctgktttagt tgkcttctga aaaaagagtc gttccctctg 960
tttctgtcct cagatgatgt ccctycccct acctgtaacc tttctttgac ataattgktc 1020
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aaaatgcccg tgaagagaag actgaatggg gtaatgaatt taatgagtct ggcaaaagtt 1140
gcaaantata tgcaagtttg ncctatcgnt ttataaatgt agnggttcat tgggattatt 1200
tatgctaggg tatattaaag ttgaaaaagt ctggggatna aaggncctna tccatggccc 1260
agaatatgaa tggcaagcaa atctttgggc aagaaaattt gnaacttc 1308

<210> 60
<211> 1121
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (4)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1114)
<223> n equals a,t,g, or c

<400> 60
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gttttactgt gaacactttt gktatttgtt ttgyaccctt ttttgtcttg tttttctgtt 180
ttagttgtct tctgaaaaaa gagtcgttcc ctctgtttct gtcctcagat gatgtccctc 240
cccctacctg taacctttct ttgacataat tgttcatatc aatgaaggyg ctgaccagct 300
caatacaaag ttaagcacaa gatctaaagc tcttgaaaat gcccgtgaag agaagactga 360
atgtgttaat gaatttaatg agtctggcaa aagttgcaaa ttatatgcaa gtttgtccta 420
tcgcttataa atgtagtgtt tcattggatt tattttatgc taggttatat taagttgaaa 480
tagtctgtga ttaaatgtcc tcatccatgc acagaatatg aatggcagca aatctttgtg 540
caagaaattt gaaacttatt gggaaaagcc tcccagtaga ttaattgttc atatcaggag 600
atttagggta agtcatgggt tgaggtgtca gatagtaata tctatttgtt ttgtacatgt 660
atatatctag gaactttgta acaacacatc tttaataatg ttaaaggttt tttcattttt 720
aatattttaa actaaaaact gtacttcaat ctcagtttct aaaattaaaa ataatttata 780

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ctgatctata tatttttct ttttgaaaga tttcattaag actgatgggt aactttcaaa      840
tgagggtcat gtacaaatat tgggatgcat gagatcccat gatcttgtgt attgagctta     900
ttgttgaaag ggattttga aggacagaac aattactcca tgatgaatct tcctttctct      960
gccttctgag caccgtcttt aatttccata tcttcaagtc ttgaagaagt tgatgttaat    1020
tgaagaattc acttgtctgg ttgaaataaa gcctgtttct gttgtgawra aaaaaaaaa     1080
aaactsgtgg ggggcccgga cccaattgcc ctangggagc c                        1121
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<210> 61
<211> 2013
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (49)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (54)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (685)
<223> n equals a,t,g, or c

<400> 61

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ccccaaacag aaaattgccc agacaaggcg ccccgaccct ccgaccgcag cgttgaatga      180
gccccagacc tttgattgcg cgctgtgagg cccttggttg tggtgcgagg cggctgccct      240
ggtgggctct ggccatggcg ctctgtgcct gcggccgatg cgttgctgcg aacagcatag      300
gtgagactct gccgagtgag gtataggtct tctcaccacg cctgtactgc agacacggtc      360
gtgtagagta gcagctgatt tgacctgtcc cagattttaa gtgatattcc aaaagggact      420
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aattaggaaa acagctcccc tcccctccag ccatgtaagt cctcctgatt ctgtatcaca      540
tgagacacca aaaactggaa atgtagtcac acccagtaca gtaccatttt tatgaattta      600
aaaatagtct tataatttta attgtttgtg ggtatttttc tactggtata aatagcttct      660
aattaataaa tcgatcaaag gttgntactc agtatacatg aaatttttg caggaaaaga       720
aaccagaaat acagaataat tcatagaaat catggrtctc aagtatttgt tcaattagct      780
ttggggaaga aatgccaaat ccactttgag gatgagtctt acgacacaga tccacccaca      840
kgttgctctt atctggaatt tgggacaaag caatgcacag gccctcattc ctgatgtggc      900
gccctccaga catggctgcc caggaaggac ggccacttta gaagtgggac gtatcaccag      960
taagcttgaa tgwttaggat cgcaggtggc tactgcattc tgccgttcgt gaccgtgttc     1020
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aaggaacaga acgcaggcat tcaaccatga cgtctgcaca gtgttagtaa catgtcattg     1680
tggtttcgtt ctcactgtgg taaaatttat ttctgagcac ttaaactgtg ttgcataccc     1740
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ccagttgtag ccaactacca ctgctaggcc tcaatgtaaa ttcagttgaa atttgcaatt 1860
ctatcagcaa tttaatgtat tgaattcaga tcatcatttg tcattttaac cgacaaccac 1920
ccaataaatt tactctgcag ttctgaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1980
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa atg 2013

<210> 62
<211> 3387
<212> DNA
<213> Homo sapiens

<400> 62
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tgtcccgaat ccctgcagtc tttctgtagg ttgcggcaca acgccaggca aaagaagagg 180
aaggaattta atcctaatcg gtggaggtcg atttgagggt ctgctgtagc aggtggctcc 240
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agaatcagct cttgtggcct tgaagtggct gaagacgatc accctccaca ggcttgagcc 420
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aaggagaaca aagaagttat tcttaaactt ctggtcatat ttgagaacat aaatgataat 1440
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ggaagtaatt gaataattgc ccaaagattg tatgtatgag gctgttcatc ccagcactgt 2640
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aaggaatgtt cacacagtag gatattataa gtatgctgat ggaaatctat attgccagga     2760
aaagctattc attatgcgtt gtgaagtcag aaagtaaaaa agggtagata gaagtattcg     2820
aagtatagtt ccattttttg agactaataa aacatatgtt taaaaggaca ctaaaaactg     2880
gagttataga tatccagata gaaacagtag ttatctttgg gtagaagaat aatgagtgat     2940
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ttttaggacg gttttgaaaa agaaaagtgt tgggaatgaa gcaagtgatt gattggaaaa     3060
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aatgatagat aaatggtgaa gataaggctt attgtgagga ttcagtgagg taatatatgc     3180
aaagtactta caatgttctg gcacatagta attaattaag aaaatcgagc acccttaatt     3240
acctagaatg cagggttgtt agtttttttgg ttgacttttg ttttgctggg gcattctgcc     3300
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<210> 63
<211> 1420
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1355)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1375)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1388)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1408)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1419)
<223> n equals a,t,g, or c

<400> 63

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aggcggaaca aatggagtcc atgccccgag gctgtgccct caagctggcc tagggtcaga      180
gaagggagga gatggtagca gcagaaccac agcacaccac tgagcactgg aaagagcaag      240
agctgaaatc ttaggttatt ggatatgcct agagtcagaa aagccttggg aaatttttgg      300
tgatcgattt ttcctgtgaa gggggaagct gctgatggag acttgtatct gcagtacttc      360
aggttcccag ctggctcccc atccccaccc tataacatga ccggcgtaca gaaattaatt      420
ggccgactgt taccaccact agtctgtccc tggctagact agacagatgg gtgtgcccct      480
gggctcagtg gtagcctacc cggggtgatg gtggggttgc cagctgtart ccarctgttc      540
tggggcctgt gtttgtgcac ctgtggtctg tatcctgccc cacaragctg gctgtccagt      600
ggaraataca aagtgacatc tggtgctccc tcagaaagaa tgtggcccca gaggcatgcc      660
agtggcttta gactctctgg gaggacatgt ttgagagcaa cagccccttc cccatctttt      720
cctttcttct cagcagttat aaatctctct gcctgcagta artagagtga gtggctggtg      780
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asgargtttg ttgatgacac tgacaatatc tggtgactcc ctccagatgt gcactcagca     840
gtgagctggg cagaggcgtg ggaaggacgt tggctgggag cagacggcct ctgcaaatga     900
atctctagtt tcctgcaacc tgggagtgtc cccatgagtt ttactttcct actttatgaa     960
gtgaacctgc tttctttggg atttcatgta ctcctaatgc tttttctctc caggtatttc    1020
tttcctgcta catattagga ctcccaaggc aagagtgcac ctttatttag acctgggagt    1080
aaagggaaag agttgcctgt ctttcgtgtg ttttcagctt tggctctgtg ggatcagtta    1140
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accttcctca gcctgccttt tggaaaggtt gagaagctaa aaagtcaggt aagagaaggc    1260
agagatggac ttcagaacct gatttttaaa ttttaaggca aggctggctg ccactggtga    1320
gagtgatgca gcagcctagc gtgtttccca gcagnagggt ttagtctttt gcccnaagaa    1380
gttcaccncg tttattgagg gttgctantc acagtggcnt                          1420
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<210> 64
<211> 1045
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (940)
<223> n equals a,t,g, or c

<400> 64

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gttcaaattt gtgcttttat acttttgtat tgttcactta gtgcttttgt agtaagggga     180
tgagttacca agaactcaca gactgcccaa aatatgacga ctgtgcattc atataaaaaa     240
agttcagtga gtctgaaatg agccatgggt gtatcttgat aataaagtgc tatttggcaa     300
agtagaagta tggcaggcct ataaaaaaat tcccattttc ctaatggtat aaaaaaaatc     360
caacacagag ccacagatgg ttatctgata ggtaaacact tatgacctct taacactccc     420
aagtttgtgt cmaatataat gtacctcttc gtttattcca aaaggagtgt acgtatatat     480
ctatatgaaa atttggggag aagcacatgg gaagaaagca gacaggtaag tttgtccttc     540
ctatggagtg cacaggggtt gaggggaact ggctaaagaa ggaaaaggca gagaagaagg     600
aaactcacca aagaacattc aactttttct tttgtttaat gattacttcc aggaaaggag     660
aagaaaagcg ggcaggggtg gaagcaggat ggagtttgag agaaagacag aaagagacag     720
aggagaaaaa cagaatggaa atactggagc aagggcaggg tgtgaagatg gagagtggag     780
cctggcccac actggctgtc aggaagggaa ggagagaagg caaacattgc catcattcca     840
gcatctcaga tttttcaccc maatgcaaca acaacgaagt aaacgctgct ttcattacaa     900
tactctccca agagactaga gcaaggaggt tttactttgn acttaccctg ccgtcatctc     960
ttttaagggt aggattcatt cactgtctat aataacttag tttaaaagag gtactttccc    1020
tctaggaagc tgttcagctc gtgcc                                          1045
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<210> 65
<211> 786
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (610)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (663)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (698)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (706)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (737)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (740)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (762)
<223> n equals a,t,g, or c

<400> 65
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aatgcattca gaatcttcag agtcaggtga aaagctttgg ccatgattgg ccttggcatt 120
ggttgtgctg gacagcggga ccaggcgccc ccttacctgg ctccccccctc ccaggagccc 180
ggtgatgctg cgaaggctgt gaacagggga ggcggcactg tgggggctgc cggcagccgg 240
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tgggccctcg ccctggggct gggcctcatg ttcgaggtca cgcacgcctt ccggtctcaa 360
ggtaggggaa gtctggtggt ggcggtgggg agggagcgaa aaatgtaaga gaccagttgg 420
gctccaacag aaagaggcat caggggggctg ggatgggggt caatggggga aggccctggg 480
gtcaataggc gggagccttg cagccaactc cctggatttc gggggtcaag tgaggccagc 540
atcacttgct ccagcagcct aacagccagg acacaggggt ccaataagac cagggcccac 600
cccargcctn tgacccttac ccacagatga rttctgtcca gtctggaaaa gctatgagat 660
cgnctttccc amccgcgtgg accacaacgg ggcactgntk gccttnttgg caacttcttc 720
ccggaagcag cggccgnggn accgggggggc cacaggccaa tnccggcttt ttttacaaag 780
gggctt 786

<210> 66
<211> 237
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (89)
<223> n equals a,t,g, or c

<400> 66
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tcctgctgaa cctgacccgc agcttccgnc tactggcagg gcacgtctcc gtggagtact 120
ggacacggga gggcctggcc tggcagaggg cggcccggcc ccactgcctc tacgctggtc 180
acctgcaggg ccaggccagt agctcccatg tggccatcag cacctgtgga ggcctgg 237

<210> 67
<211> 233
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (137)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (232)
<223> n equals a,t,g, or c

<400> 67
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aaattttaa tttatttttc atagagatgg ggttccacta tgttgcccag gctggtctcg      120
aattcctggc ctcaagngat cctcctgcct cggcctccca aagggctaag attaaaagca     180
tgagtcagca tgctcagcct ctaatttctt tttattttaa aattatatat gng           233
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<210> 68
<211> 797
<212> DNA
<213> Homo sapiens

<400> 68
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taccggtccg gaattcccgg gtcgacccac gcgtccgata tcactcagtg tttgtgtttt      60
cttctctttc tagagttgta aagatgtggc acctgtggag aagactatta agttgcttcc     120
cagtagccat gttgcaagac tacaaatatt cagtgtagaa ggacaaaagg caattcagat     180
caaacatcag gatgaggtta attggatagc gggtgatatt atgcataatc ttatttttca     240
aatgtatgat gaaggagaaa gagaaatcaa tataacatca gctttagcag aaaaaattaa     300
agttaattgg actcctgaga ttaacaaaga acacttgcta cagggtctgc ttcctgatgt     360
gcaagtacca acatctgtaa aagatatgcg ctattgccag gtttcattcc aagatgatca     420
tgtgtctttg gaaagtgcgt ttacagtaag accacttcct gatgaaccta aacatttaaa     480
atgtgaaatg aaaggaggaa aaacagtaca gatgggccaa gagcttcaag gagaagtagt     540
tataataatt acggatcagt acggaaatca gattcaagca ttttcaccaa gttctttatc     600
ttctttgtca attgctgggg ttggacttga tagctcaaat tttgaaaaca acctttcagg     660
aaaacacaca gaagtataag tgtaagaggc atcaaattta ttccaggtcc tcctggaaat     720
aaggatcttt grtttacttg gcgtgagttt gtctgacttt attcgagtgc actaattctg     780
gacctyctgc taaactt                                                    797
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<210> 69
<211> 1514
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1472)
<223> n equals a,t,g, or c

<400> 69
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aagtatcata gaaggaccta taattaagtt aatgattctt ccagacccag aaaaacccgt 180
tcgtctcaat gttaaatatg acaaagatgc rtccttctta gcagggggtc ttttcactga 240
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aacatttagt tgtaataaaa taaaagataa tgacaaagaa gatggctgct tctatttcag 420
ggataaagta attcctaata aagtggggac atattgtatc cagtttggtt ttatgatgga 480
taaaacaaat attctcaaca gtgaacaggt tatagttgaa gtcctgccta atcaacctgt 540
gaagttagta cctaaaatta aaccacctac accagctgtt tcaaatgttc gctcagttgc 600
cagtaggacc ttggtcagag atctacatct tagtatcacg gatgactacg acaaccatac 660
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gcttcttaat gaaatgaaat gtcaagttga agaagcaaga ttaaaagagg cccaattgcg 1080
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agcacttctg aaaagaaagc tatcagaaca agaagaactg aagaaaaaac ctagaagatc 1200
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agcacaaatt gaagatgata gagctgcgat ggttatttct tggcatctgg caagtgacat 1320
ggactgtgta gtcaccctaa ccactgacgc tgcacgtcgt atctatgatg aaacccaagg 1380
tcgtcagcag gtgttgcccc ttgattctat ttaccagaag actcttccag attggaaaag 1440
atctctacct catttccgaa atggaaaatt gnattttaaa cccattggag atccagtctt 1500
tgctcgagac ttgt 1514

<210> 70
<211> 529
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (4)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (6)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (9)
<223> n equals a,t,g, or c

<400> 70
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tctgtcgtct caacctttgg tgaatgacgc aatgagtcat tacacttacg tatgggagtc 120
atacctagtt tttcctctat tagtttgaga tttttctcat gttcatcaag ttcttgcttt 180
ttcttcctca tatccggagt gcgaaggtac tctaattgac tgttaagatc cttattcaca 240
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atcagacact gttttggaac tggagctcca aataccattc cccgaagttt atccattgga 360
ggagctttat tctgaaggcc cccaaacttt ccattacttc gaattcgatc tccatctctg 420
gtcagcagtg taggacagtg tgtaatttta acaacctctt ttctataatg attggccgca 480
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<210> 71
<211> 960
<212> DNA
<213> Homo sapiens

<400> 71
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ccacattagc acattttcct cttacacatt tgcaaggtag tgacaaccaa agggcataat 180
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aaatactcag tctggtaagt caccctcttg gtcttctgaa acagagatgc agaaagcagg 360
gcattcttac tgagaccaat ggcctcttct ctctgtcacc tctcatacat ctgttttggt 420
tataatccta ttttgtcttg tagcttctcg tctcattctt ttaggaggaa ctggacaatc 480
tgtcgtctca acctttggtg aatgacgcaa tgagtcatta cacttacgta tgggagtcat 540
acctagtttt tcctctatta gtttgagawt tttctcatgt tcatcaagtt cttgcttttt 600
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gtctagtttg cacacagcag aacgatactg ctgaagaaga tctatttgtt cccctaagat 720
cagacactgt tttggaactg gagctccaaa taccattccc cgaagtttat ccattggagg 780
agctttattc tgaaggcccc caaactttcc attacttcga attcgatctc catctctggt 840
cagcagtgta ggacagtgtg taattttaac aacctattgt aaataagtga atagtaagta 900
gtgagcaact tatttcaact gctccaaaaa aggagaaata atttaaagtt tctgagatta 960

<210> 72
<211> 2393
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1324)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (2336)
<223> n equals a,t,g, or c

<400> 72
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cctcccacta tggggaaaag ggttgcttgg aggacctggg gtggcagccc cccgaccaga 180
agcaccctat tttatagata gcagtctgaa tggtagagag tcctgtgtgt gggttgttag 240
aaggttggtt cttcctgtta ttctctctgg ctttcctttc aacccactta ttttcagagg 300
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tcaacagtat tggttaagtc acaacgacga agtgctgaga aggttatggg taccaggaag 420
aaacaaacag aagtcttaag attagaatga gaaccaaaga gaatttaacc ctgccatttt 480
tttttttta acaccaagat cctaagtaat tccaaatgcc ttagatatca atgaaagcta 540
cacaccattg agatgggcaa aattctttct ctacaaaggg agtaatcaag taaatacctg 600
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tcctgtcctc tgtgccagga tttggtctta gaatccatgt cagattggtg cttccagaca 960
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aagaacagaa tacagacata aataagtcac ggcccatgac cttgaagagc ttacggcctt     1200
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tctgtagaat gaatagttat gttttgatat gactgatact ttctttgtta aaaggttgta     2160
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caagaatgcc tatgaaatac ccactataac agcattcctt ttgtgtttag aactatagaa     2280
gaaaatgtct tcaaataaag tagttttcag tcaaaaaaaa aaaaaaaaaa aactcnaggg     2340
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<210> 73
<211> 748
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (669)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (682)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (685)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (724)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (728)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (734)
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<223> n equals a,t,g, or c

<400> 73
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gttcttttga ttttgctttt agataccaac ctttgcagac agattagctc acatagtttt 120
gaatttagtg ggaaccaacc ccttgtattt tgctgtattt ctagtatctc tgctaaattg 180
gtactagatc aggcaggatg acttaatgca acagttctta aacattttgg tcttaggacc 240
cctttatgca tttgaaaaca tcattgagga tcccaaagag ctttttctgt aatgtgaatt 300
atatctgttg atatttatca tatttgaaat taaaactgat aaaatttaaa tacaaaaatg 360
tgtaaccata tatcctatta gccatcagag tgatgccaac atcaaacatc atatagcttc 420
tggaagactt cagagcacat ttattagaga atgagagtga aaaagtcaaa taatttattg 480
gtgttatcat gacaataatt ttttgatatg tggacccccct gagatggtct tagggacaat 540
gctaaagact actgktttaa agtatggcaa agaagraaac caccttatgg gaagrtttca 600
tgagtctttc tcaaagggac tcccttggct aaaccatcct aatccttcca tgggacctaa 660
gccattcanc ctttwaaccc anggnccttt taaggcccaa ttggtaattt acctgggggga 720
gccncctncc ctcnaaaaat gccaggag 748

<210> 74
<211> 991
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (11)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (12)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (23)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (880)
<223> n equals a,t,g, or c

<400> 74
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caccttatgg gaagatttca tgagtctttc tcaaaggaac tccttgctaa acatctatct 180
tcatggtacc taagcatttc acctttttaac caggacttta ggccattgta ttactgggag 240
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atttaaattg gcttattaaa tggcttttca gttacttttc aggatgttga gatacttgga 360
cttaggatag gtatgtttta tggggaagac ttgggaggtt atagagaaat aaaggggaga 420
aggaaagggt aattgagagc atgcctctct gctgagaagg tggctccatg tggaacacca 480
cagccttgct ttctcctcta tgtctcctgc cctctcaccc tggctggctg ctcaccaacc 540
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ttagaataac tcagtcatgt atttctcttc ttagtaaaaa ttcagaaaaa ttccagagta 720
gtgcaaacct gcttagagag aggtgaaaac agaagaagac tgttgtgttt aactgaaact 780
gaaactgcaa atgaaggggt tttttgwca ttatatgctt tctttttggt ttgkattcct 840

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<210> 75
<211> 2751
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (2642)
<223> n equals a,t,g, or c

<400> 75

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cgcgctctac gcgagcctgg accggaccgc caccccgatg tacgacggcg ccgacacatt      180
gagcgtccgc gttttcgaac cgtaaccgtc ctttctccag gggaaccacc atgaagatcg      240
tcccgctcac cgcggccgtg ctggcgctgg tgctggcgcc ggccgcccat gcgcarccgg      300
ccaacaaggc caccactgtt tcgccgaccg ccgccgcctt cctggcgcag ttcgccaccg      360
agggcaacga cagcgtcagc tgggcccagt tcgaggcctt ccgcaagcag cgctacgccg      420
acaccgaccg caaccaggac ggccacgtcg acgaacagga atacgtcgac gaatacctgc      480
agcgcttcga cgtgcggctg gccgatgcac gcgccggcca cctgcgccag accgacaccc      540
gtttcaaggc actggaccgc gacggcaatg gggcgatcag ccgcgccgaa tacgatgccg      600
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cggcgtcctc gcgcgatccg ctgaagatgc cgacctcgca taccgccaac ggcatgttgg      720
acctgtacga ccgcaacaag gatggcgcgg kggatcgcga ggagttcgac gcggtgcgcg      780
cagcgagctt cgccgmcacc gacaccgacg gcaacggcac gctgtcgttg gccgagtaca      840
ccgycgaatt cgaaggccgc ctggaccagc agcgccagcg cgtgcgcgcc gatgccgagc      900
gccaggcacg cgtgcgcttc gcctcgctgg acaaggacac cgatggccgc atgacgttcg      960
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acgctgaaac cgctgccgac gccagcacgc tcgataccgt gcgcgtgcag gccgagcgcg     1380
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cgggctactc gggtgccaac gacgaaacca tgggcacgct caccttcgcc aaccgcaccg     1860
gcatcgtcga atcgatgctg gtctacaccc gccgcgaagg ccatgaatcc gagtcgtggt     1920
atgacaccac gaacgaccgc atcggggtcg gccgccgcac gccggacccg gtcgacagca     1980
cgcgcgacaa cctgctcggc aagctcgacc tgcagctgga cgaggscaac acgctgggct     2040
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cgggttatct gtcgcgcaaa ggccatgaca ccaacgaccg tgaccgctac ggggtgaact     2160
accagtggcg cgccgacacc gcgctgttcg atacgctgga tgcgcaggtg gaccgccagg     2220
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cgccgtgcct gsgcagcgag aaccgctcga ccaagcagac cctggaccgt gccgcggccg     2340
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ggcagcagcg cgacatcgat ttcaccgccg tcgatacgcg ctggaatgcc gccggtgcca     2460
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gctatgaccs gttacgacta ttcgcccgca agtggatgcc acctttgtgg atcggaaccg 2640
gnaccgtgcs cgatgtgaag cttcgctttg gccgarctgg aaggcccggg ggccgaatac 2700
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<210> 76
<211> 774
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (13)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (711)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (713)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (717)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (740)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (764)
<223> n equals a,t,g, or c

<400> 76
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accggcggca ttccagcgcg tatcgacggc ggtgaaatcg atgtcgcgct gctgccaggc 540
caggccgtag accacatcat ggcgcgcgcc tgcggtggcg aacaccttgc tgaagtcggc 600
cgcggcacgg tccagggtct gcttggtcga gcggttctcg ctgcgcaggc acggcgtgcg 660

ccgccgkgac agccgctkcc ggccacgatg gggttttggg gttggaaatt ngncccnaac 720
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<210> 77
<211> 655
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (4)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (5)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (7)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (10)
<223> n equals a,t,g, or c

<400> 77
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gttcttgctt atctaatttt aaacgaatat gaaagacaat ttaagtgatt ttgtacattt 240
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agtttcagag taatgacaaa ccaaacacac attctaattg tctacttgta caatttgttg 420
ggtaggtact atagggggaa gaattaaata tacatgagga agagaaaaaa tctttgccaa 480
gaatttcagt tattatcact tatggtttac ccatattgtc ataaaagtaa gaaaatggga 540
attctaattt tctgtcacaa aataaagatt atataaaaat aaaaaaaaaa aaaaaaaaaa 600
aaaaaaawta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 655

<210> 78
<211> 748
<212> DNA
<213> Homo sapiens

<400> 78
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atcttggact ttggagaacc atttcaatgt aaagattcaa acctttcttt tgcttagtaa 180
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tgtccttggt tggttttatc tcacggatct gccatctaat ttctttttaa atctcttcac 300
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gctgatttga tgcttygcag taactctttt tcaccttcat tgagtgktta cctttgckca 420
ctttgktttt ctttggkttc tagtaaatcc agcaagtaag ggctctaatt gtacttcctt 480
gggcatttat ttaaaaaaaa aaaaatgaaa gtactttatg tctttcagta gtgttgcctc 540

aggaaagaaa ccatacttgt tctgattatt cacaagggct ttcttttagc ttcctcaccy 600
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agcaaactgg taaaatctgg gggaacat 748

<210> 79
<211> 1314
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1032)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1098)
<223> n equals a,t,g, or c

<220>
<221> SITE
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<223> n equals a,t,g, or c

<220>
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<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1307)
<223> n equals a,t,g, or c

<400> 79
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aactggagca cggagctgca gccggttggg ccggtgtact ttcccgctct ggaaaggaag 180
agaaatggaa gtgagaaagt tgagcatttc ctggcagttc ttgatagttc tggttctgat 240
cctgcaaatt ctgtctgcgt tggattttga cccatacaga gtcctagggg tcagccgaac 300
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caaaaacaaa gatcctggag cagaagacaa gttcattcaa atcagtaagg cttacgagat 420
tctttcaaat gaagaaaaga gatcaaatta tgatcaatat ggagacgctg gagagaacca 480
gggctaccag aagcagcaac agcagcgaga gtatcgcttc cgccatttcc atgaaaattt 540
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cgaaaagtat ttattgcact tttcacatta tgtgaatgaa gtggttccag atagcttcaa 660
gaaaccctac ctcatcaaga tcacctccga ttggtgcttt agctgcattc atatcgagcc 720
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acaatttgta gaaagtcttc ttccagggaa cttggtggag aaagttacaa ataaaaatta 960

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aacgcccatt gngccactgt tatacaagtt gactgccttt gcatacaaag attatttatc    1080
atttggatat gtatatgngg gtttgagagg gacggaagag atgacaaggc ggtacaacat    1140
caatatctac gcccctaccc tcttggcttt aaagaacata taaacaggcc tgccgatggt    1200
atccaggccc cgaggtatga anaagcaaat cattgacgac ttcatcaccc gaaacaaata    1260
tctattggca gncaggctca ccagccagaa agttggtcca tgaactntgc cctg          1314
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<210> 80
<211> 612
<212> DNA
<213> Homo sapiens

<400> 80
tcgagattca cccaggtaga gggaagagtc aacatcactg cacgacccta tggcttcctc      60
ctcttcgact gtcttttggg gcttgaagag gcagaagtat ttcttgtggc cattcagagc     120
cagtacataa ccagtgtagt cacgctccat gaaatgctta tcatattggt ttgggattgg     180
agctgcatct tgagcaaatt ctagtaagta ttctagccat tctctgtgtt tatctagact     240
caggaaggag aagtgtaggc agctgctccc agtaaatgtg tagacctcca aagcaaattt     300
ctgtagtagg ctggtcttgg tagaattcga caggatgagg accacattca tgtggcctgg     360
cctcagacgt accaagttac tggtgtatgt tacatctgtg agttcagtta cctccacaaa     420
gcctttttta ggaatcttgc tgctgttttc tttggtgaag cttggctcag ttttcccagt     480
cttctcttgg gcttcctctt tttctggagg gcttgactct cgctcatcat tagagtcgct     540
gaaagcctga acgatgacag tgccgaagag gatgaagagg gcagagaaga tcagggacag     600
caggggcatc at                                                         612
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<210> 81
<211> 733
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (716)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (720)
<223> n equals a,t,g, or c

<400> 81
gatccctaaa caagtctttg tgtggacata tgcttttact tctcttgagt aaatacctag      60
gagtggaatt gctgggtatt tttaactttt taagaaacaa gctgtttctc aaagtggttg     120
tatcacttga aatcctacca gcactgtatg aactccagtt tcttcatatc cttgccagcc     180
ttgatatggt cagtctgttt aattttaggc tggtggcagg tgtctagtgg taaagtagct     240
cattgtggtt ttatcttctg tttccctaat aactagtgat gttgagcctg ttctcacgtg     300
cttatttgcc acctgtattt ttatttattt atttgcttat tttgagacgg agtctcactc     360
tgtcgcccag gctggagtgc cgtggcgcaa tcttggctta ctgcaacccc tgccttctgg     420
gttcaagcga ttctcccacc tcagcctccc gagtagctgt gtctacaggc atgcgtgacc     480
atgtctggct aatttwwgta tttwwagtag agatggggtt tcgccatgtt ggccaggctg     540
gtctcaaact cctggcctca agggattcgt ctgccttggt ctcccaaagt gctgggactg     600
cagatatgag ccactgggcc tggtggcccc tgtatataww waaatgtctc aaaatggttt     660
acccattttc aatttggtgt ttggcattac tgaattgtcc tttaacctag aatcanggan     720
aaaatttgaa aag                                                        733
```

<210> 82
<211> 594
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (458)
<223> n equals a,t,g, or c

<400> 82
cgtgccggtt tttttttttt ttttttcttt ctttctttct ttctttcttt ctttctttct 60
ttctctctct ctctctttct tccttccttc cttcctcttc cttccttcct ttgttccttc 120
gttccttcct tcctttctcc ttccttcctt cttttctttc tttctttcct ctctttgttt 180
ttttgagacg gagtctcccg ctgtcgcctg ggctggagtg agactccgtc tcaaaaaaaa 240
aaaaaaggaa aaaaaaaatt tcaattcttc acacactctt ccagaaaagt gaagagggca 300
gtacattttc caactcattc tatgagaccc acattaccgt gaatccaaaa ccagacaaat 360
agcctagaag gtaaaaaata agtacagacc aatgtcattc atgacgacag atgcaaattt 420
tcttaacaaa ataatccaac atggattatt catcatgnaa atacatcatg acaaagtaag 480
atttaatctg ggratgccaa gttggtctag gattcctaca atcaaaaatc aaccaatgta 540
atccagcatt atatggaaaa ctgtatgact gtcttactaa atgcaggaga aaaa 594

<210> 83
<211> 1484
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1484)
<223> n equals a,t,g, or c

<400> 83
gcagtgatat gcctgttttt gtgtgttcca tcggactttg tttccttttt tcgattttgt 60
tattgtttcc acctttttcaa ttttcttaca tttgttggct ctctcaagct tctgtttatt 120
ccccttctcc ctcccttagt aatttggaag ttctactctg cttatcaatt cttctgatga 180
ttatctttcc attccttata agcataawtm acatttwctc tattggtaga ttaagcacac 240
acatgggcgc acacacacac acacacacac acacacacac acacacacac acacasgtgt 300
gttactggcc tttactccta atttctcagg agaatgaacc atttagaatg tttttacctc 360
ttcattctgc cctcacccaa aatttctgtt cttagaaaag aacagatctt tttttttctg 420
agattttttt tttttttaat tagaagagac gctggaagca tgatgacagc agcgtagttt 480
tcactctctt gttatgccag catacagaca gagcaaccag atgacaaaat caaaacccat 540
gggtaatatt tacggtgagg ccagtagaca cagtgtcccc agtgtggcag atttctgcca 600
gatagggctg ccagctgtat ttccaaccct gtatgtgttt gccaccctcc catcaagaca 660
tggtgcctat tttttcatcc tttgtgtctg ggctggcctt gtgttccatt tgaccaataa 720
ggtatggtag aaatggcatt ctgggacttc tgagcccaac cttaaaagac attgaacttt 780
tgctttagtg agaggggaat caagataccc tgtaaagagg tctgtgctgt ccttctgaag 840
acagaggtcc tggaagatga gacacttttt aaaaataggt catgcagcgc tgtggcagcc 900
acccagctag ctcagttggt agagcatgag actcttaaat ataggccatg tacataactg 960
aggccccagc tggcrgctag cactgaggtc tcagtcgtat catgaamtta tcttgcatcc 1020
tccagtacca ttccagcttc tccaggcatc atatagagaa gaaacaagtc tgggcgtggt 1080
ggttcacacc tgtaatccca gcactttggt aggccgaagc aggcagatca tctgaagcag 1140
tgaagcaagg agtttgagac cagcctgggt agcaaagcaa gacctcatct ctactaaaat 1200
atattagtca ggcacagtgg cacatgctgg tagtcccagc tactcaggag gctgaggtgg 1260
gaggattgct gtgcccagga ttttgaggct gcagtgagct atgatcgcac tactgtactc 1320
caatggtcaa cagaacaaga tcccacctct taaaaaaaag aagaagaaga agaaacgagc 1380
catccccact gagccatgcc cagattcgtg agcaataaaa tagttattaa tccactaaaa 1440

aaaaaaaaaa aaaaaaaaaa aaaaaaaaag ggcggccgct ttan 1484

<210> 84
<211> 774
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (618)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (715)
<223> n equals a,t,g, or c

<400> 84
gtctacctcc gggctgaaac gtcaccatgc ctccccacag acagacggat ggacagatgg 60
gcctccctgc acctgctctg tgggtgtggg ggctcctgct cagcagcagt ttccagaccc 120
ttctccctgc tttccccaag ccacccgcct tgaatctggg gtgctctacc agacccatcc 180
cctcatttct aaagatttga gccactagtc gtgtccctct ccctcagaaa tgccttggtg 240
acacttggct gctttcaact cttccaccca tctgcctctt ggtctcatct ttaccttctg 300
ctaaaggtcc tgacccccac ccccgccacg ccatggggca ccccatggtg gtgcgtcctt 360
gggagcagct ctgtcccttt ccccgtggcc tttgccccgc ctcctatgac ttcgattccc 420
acctgtcccc gacccctggg accactgacc gggcccgatc accctgtcac tgccctgtca 480
tctgcttacc ccacacggtg ctctgctgac ccaggtcttg ctgtctccca ayagccccac 540
gaggcttycc gtcgctcctg gacactrmag gctgagcccg ctgccccgcc gcctccatga 600
ggaaggcttt tcctctgnga gccccaggcc accctttccc tcctttaagt aattacttaa 660
gtcccttgcc agggccctcc cagtaccctt tctaaagaca cccctgcccc agcangctgc 720
aggctcctgc tccactttcc tctcaggccc tcgtcgctgt ggtgctgcct ttga 774

<210> 85
<211> 1396
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1187)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1325)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1327)
<223> n equals a,t,g, or c

<400> 85
cctcgagcca tgtgtccagg tggggcagat catggagggc gcccaggtgc ggtgctgagg 60
ctgagcatgc actggctggg gagggtgggc agagcaggag gaaatccctt gttctccgga 120
gctggagagc cagaaagagc cctgcagcct gggcctcatc atcacacctc gccctcaagg 180

cctccaggca cagcatccac tgccagcctc tgctcctgcc tctgagggtc tgtctccaag 240
gtcttttggg ggctgcccca gctcccccaa cacagacagc accagagctg ggcccacctg 300
tgaggtgctg agttccccat ctcagagact gtgcgagcag aggcagggag gcgcttgatg 360
tcggatggag aaaggacagg ggaggggtgt ggggctcagg gccccgccag gtgaaggaac 420
aagcttggaa gggtgtcctt atgtctgaga gttggggaga cacccccaag ccccagatgg 480
rcctcgaatg ccaggcaggg ccaagctggg cccagaagtg ggawggwtcc cttggctgcc 540
ccaggaatgc aggttccggg gcaagaatcc agccctggct ggttgaagtc catcccaagt 600
ctccctcccc acgaggctcc tgcagatgcc aggaatgggg ctggattcca gattccaagc 660
ctggcsgccc agcccctatc tgggacccca gcccagagcc cccaggcctg gcctccaacc 720
tggccccagc ctcaggggag ctgaattcag agaatcctgt cctaggagcc agaagcgggg 780
gagggaggra gggcggccct gtcctgggtg caggcccggg ggctgggggy tgcccgcccg 840
tctgggtcag ccgcagctgc aaaccggccc tggctgagtc atggsgcctc catctccagg 900
gcctggcttg aggtggggaa tagcagtgag gttggacatc caggcacctg agggtgggca 960
gggctccctg cggctggggt ggccagtggc acctggctgt tgccccccctg caccccagcc 1020
ctttggcccc caagtctctg ccacctccct ggggttctgc tcccatattc ctcacaccca 1080
gcacagaacc cagcatgtct cctgtagaca cctgcatata aaccctgact cacacacaca 1140
cacacacaca cacacacgca cacatgcagg ccaggctcct cggccangtc accctaccgg 1200
cagagctcta gacatttctg gcctctggtg actattcttc aggcagctca ccctgcaagt 1260
ctttattgag cacctactgt gtgccaggca gtggtacagc aagggcagaa gccccacctc 1320
caagnanctg aacccctgcc gtggcagaga cagaaaacaa aggcagcacc acggcgacga 1380
gggctaaaga gaacgc 1396

<210> 86
<211> 522
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (31)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (437)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (475)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (501)
<223> n equals a,t,g, or c

<400> 86
gctctgtggt gcctacccta cgcctctccg ncgtcctccc ccgaactggt ggttacgcgg 60
gccgcgagtt accgtcccag caggcagctg attgtttaga tggagagacc acaggggaga 120
gttacctgtt gctgtgggag caatgaggag ttatggagga aaggagaggt accaagacca 180
gatgaggact gaacattaga cttcccatga ttgcagaact gtccttcgct gcagctgtaa 240
aatcgcggca aggcaggatt catcctggtg gtgggtgcag gccctgattc ccgtcactga 300
gcagggatac agcaggaagc tccacctctg agcacagcaa ggggattgtt ctgattctga 360
gtgtgtctgt ttgctagagc ggtggccaca cttccccggt tctgcaattg tggataattt 420
agcatgactt gttgggnatt gcaaatatca tgtcgtggag agtctgggct ctgtnatttt 480
ttccagctgt gtgtgtgtgt ntgtgtgtgt gtgtgtatac at 522

<210> 87
<211> 608
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (540)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (541)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (572)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (598)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (601)
<223> n equals a,t,g, or c

<400> 87
gagttttttt tttttttttt tataaagaaa agagctttaa ttgactcaca gttcctcagg 60
gatggggagg ccacaggaaa cttacaatca tggcagaagg ggaagcaaac acatccttct 120
tcacgtggcg gcagaaggag aagtgcccag caaaaggggg aaaagcccct tataaaacca 180
tcagatctca tgagaactca ctcactatta gcaaaacagc atgagggtaa ctgcccccat 240
gaataaacta ccacccatgg ggtccctccc atgacacatg gggattatgg gagctacaat 300
tcaagatgag atttgggtgg ggacacaggc aaactgtatc actcattaag gagggttaga 360
ataaaaatgc tatgacaaac atcttggaat ttctgtaggc atgaattcag aggttgaaag 420
gccatatttg ggcaaaaccc tcagatactg cctcctcagt gtgttacttt tggagagaaa 480
tgattatttc ctaataatct cctactatac caaagaattt tctaattaga ggaaaagggn 540
nggtagcatt tttcttgggt aggaggagag gnagatgctc tgatgctggt gcaaatcncc 600
nagctttg 608

<210> 88
<211> 883
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (12)
<223> n equals a,t,g, or c

<220>
<221> SITE

<222> (19)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (25)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (812)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (877)
<223> n equals a,t,g, or c

<400> 88
aagcgctgca cnctgtctnt ttccnggctt ccttgctgtg catgtctgcc tgttttctgg 60
atggttggta gygctatgtg gggtctgatg ctgagatcat cgkcactctg tttgacctag 120
actctatgtg gatggggtta tggtgctgca ggccatcatg tgatcatggt agaatgatgg 180
cgggttctca gggatgaaga gagtcagttg ctactggtcc ccagggcagg atgcacacta 240
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cactctgagg cagtacagct gaatgatctc ttggctactc tccagactgg atttgtggcc 360
actgaggact taaggactct cctttatcaa gaattgctgg tgcctgtggc agcagtgggg 420
accactgata ggtatctcta gcttaytttt tccctgtaag aatttcctct gattttaagc 480
tgattttggc aagggagaca gtgtggcagg taggaaacct cactcccttc cctgtggtgc 540
tatcctgagg cttccatgct ccacagggat ttgccctttt ctctggtgct ctgcagcata 600
tatctacagt cattttagtt gaaatatagy tatttgktat tttggtccct ttttgktgtg 660
ggtgatatgt gccaggtaac tctggtgagc cattttttttt taaacaaatc ttcatacaga 720
wtgagtatcc cctatccaaa atgcttgaga ccagaagtgt tttggawttt gktttgtttt 780
gttttgcttt tggattttgg aatagtaata antgtattat acttatttgt tgggcatccc 840
aaatcctgaa agatgggccc ttgaacagag tgtgagngaa gcc 883

<210> 89
<211> 765
<212> DNA
<213> Homo sapiens

<400> 89
tcgaggctac aaagctaagt tgctgcagaa cctggcttct gttttcctct ccaacttacc 60
tcgttacaga tcccctatat tgaacattac ccccgcaact agaaagcact aaactgcttg 120
tagtttagta tgatattgca gcttagtata ttgctatgca tgataatgta ctgcttttat 180
atccctgctc atatttctc tttctctgca tcgctaccta gttactcatc cttcaaaact 240
cagttcagag atcagctcct ccaagaagcc ttctgtggtt gtggttggtg ctctttccta 300
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gttgaagaca gaaactgtcc tcgatgtttg tcgatttgct gaacagatga tttaaagatt 420
atacaggttc aacatcccaa ttccaaaaat ctgaaatcca aaatgctcca aaatccaaaa 480
ctttttgagt accaacatga tgctcaaagg aaatgttcac tggagcactt cacattttgg 540
attttggagc atatttcaga tttgggatgc ccaacaaata agtataatac aaataaacta 600
ttccaaaatc caaaagcaaa acccaaaaaa aacaaaaaaa cccaaaaaaa aaattcccca 660
aaaaaccaac cttttccctt gggggtctcc agcattttgg atagggggg atactcaatc 720
tgkatggaag attkgtttaa aaaaaaatgg gcycaccaga ggtta 765

<210> 90

<211> 1190
<212> DNA
<213> Homo sapiens

<220>
<221> SITE
<222> (1164)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1180)
<223> n equals a,t,g, or c

<220>
<221> SITE
<222> (1189)
<223> n equals a,t,g, or c

<400> 90
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ccaggactca gggcccccaa ccagggtatc tggtcctcat ggaacagtgg aggcctcgtc 120
tctcccctcc tgccccctac tctcccccac catctctccc ctatcttgaa gttgtgccac 180
cctctgcttg gcctacaggc ccccatccaa aggtaaaaga gctgggccat gtgcaaactc 240
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tggttgggag gtgctcgatc ctgtcttcta cccctyagag gcatccctct ctcagctggg 360
agggtctggg aggttgacaa ccaacaattg gsaacaatta ccatttatgg agcacacact 420
atgtgccagg caccacattg aacctaagta cagcccaaga gatagacact ggtttttgcc 480
catyytasag gccaggaaaa tcctgtgctt caggtcgcaa gattagaaca tgtagakctg 540
gagctcgaac ccatttctgt ckgasggsaa agcctagccc ttgactccgc tcccgcagcc 600
tccgtctggg agctcttgag gctacacaag acattccagg ccctagagag actggagcac 660
cctgttcccg gcagagccct cggctctgag ctcggmccag catcaaaaca tctcacggag 720
ccccagctca tctcatgagc taccccatcc cggcccacac ctgggcacac ctcccacccc 780
agcagtgggc atgaatcagg cagggccaga gccggagaga aaatgccaag tgccatgaaa 840
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ccagagtgac ccagagagaa agctgggga agggggcatc agggaagccc cagccatccc 960
cctcccatgg aaggagtgtt gggggagcta tgcagataca gaagtacagg aacgttcggc 1020
tgcttgtatg gccaggaaaa tgggcgtagg tcgctgcgtg tctgagagcg ggtgtatccg 1080
tgtgtgcggc catgtatgtc tttgtgtgtg tcagcaaatg tagraatgtt tgccttttta 1140
tccctgtggc aaggaggggg aagngcggtt tcttgaagan aggtgggtna 1190

<210> 91
<211> 439
<212> PRT
<213> Homo sapiens

<400> 91
Met Val Pro Ser Ser Pro Arg Ala Leu Phe Leu Leu Leu Leu Ile Leu
1 5 10 15

Ala Cys Pro Glu Pro Arg Ala Ser Gln Asn Cys Leu Ser Lys Gln Gln
20 25 30

Leu Leu Ser Ala Ile Arg Gln Leu Gln Gln Leu Leu Lys Gly Gln Glu
35 40 45

Thr Arg Phe Ala Glu Gly Ile Arg His Met Lys Ser Arg Leu Ala Ala

50 55 60

Leu Gln Asn Ser Val Gly Arg Val Gly Pro Asp Ala Leu Pro Val Ser
65 70 75 80

Cys Pro Ala Leu Asn Thr Pro Ala Asp Gly Arg Lys Phe Gly Ser Lys
85 90 95

Tyr Leu Val Asp His Glu Val His Phe Thr Cys Asn Pro Gly Phe Arg
100 105 110

Leu Val Gly Pro Ser Ser Val Val Cys Leu Pro Asn Gly Thr Trp Thr
115 120 125

Gly Glu Gln Pro His Cys Arg Gly Ile Ser Glu Cys Ser Ser Gln Pro
130 135 140

Cys Gln Asn Gly Gly Thr Cys Val Glu Gly Val Asn Gln Tyr Arg Cys
145 150 155 160

Ile Cys Pro Pro Gly Arg Thr Gly Asn Arg Cys Gln His Gln Ala Gln
165 170 175

Thr Ala Ala Pro Glu Gly Ser Val Ala Gly Asp Ser Ala Phe Ser Arg
180 185 190

Ala Pro Arg Cys Ala Gln Val Glu Arg Ala Gln His Cys Ser Cys Glu
195 200 205

Ala Gly Phe His Leu Ser Gly Ala Ala Gly Asp Ser Val Cys Gln Asp
210 215 220

Val Asn Glu Cys Glu Leu Tyr Gly Gln Glu Gly Arg Pro Arg Leu Cys
225 230 235 240

Met His Ala Cys Val Asn Thr Pro Gly Ser Tyr Arg Cys Thr Cys Pro
245 250 255

Gly Gly Tyr Arg Thr Leu Ala Asp Gly Lys Ser Cys Glu Asp Val Asp
260 265 270

Glu Cys Val Gly Leu Gln Pro Val Cys Pro Gln Gly Thr Thr Cys Ile
275 280 285

Asn Thr Gly Gly Ser Phe Gln Cys Val Ser Pro Glu Cys Pro Glu Gly
290 295 300

Ser Gly Asn Val Ser Tyr Val Lys Thr Ser Pro Phe Gln Cys Glu Arg
305 310 315 320

Asn Pro Cys Pro Met Asp Ser Arg Pro Cys Arg His Leu Pro Lys Thr
325 330 335

Ile Ser Phe His Tyr Leu Ser Leu Pro Ser Asn Leu Lys Thr Pro Ile
340 345 350

Thr Leu Phe Arg Met Ala Thr Ala Ser Ala Pro Gly Arg Ala Gly Pro
355 360 365

Asn Ser Leu Arg Phe Gly Ile Val Gly Gly Asn Ser Arg Gly His Phe
370 375 380

Val Met Gln Arg Ser Asp Arg Gln Thr Gly Asp Leu Ile Leu Val Gln
385 390 395 400

Asn Leu Glu Gly Pro Gln Thr Leu Glu Val Asp Val Asp Met Ser Glu
405 410 415

Tyr Leu Asp Arg Ser Phe Gln Ala Asn His Val Ser Lys Val Thr Ile
420 425 430

Phe Val Ser Pro Tyr Asp Phe
435

<210> 92
<211> 180
<212> PRT
<213> Homo sapiens

<400> 92
Met Trp Thr Leu Phe Ala Leu Ser Gly Pro Leu Phe Leu Phe Gln Val
1 5 10 15

Leu Thr Phe Met Ile Tyr Ile Val Ser Thr Val Phe Cys Gly His Leu
20 25 30

Gly Lys Val Glu Leu Ala Ser Val Thr Leu Ala Val Ala Phe Val Asn
35 40 45

Val Cys Gly Val Ser Val Gly Val Gly Leu Ser Ser Ala Cys Asp Thr
50 55 60

Leu Met Ser Gln Ser Phe Gly Ser Pro Asn Lys Lys His Val Gly Val
65 70 75 80

Ile Leu Gln Arg Gly Ala Leu Val Leu Leu Leu Cys Cys Leu Pro Cys
85 90 95

Trp Ala Leu Phe Leu Asn Thr Gln His Ile Leu Leu Leu Phe Arg Gln
100 105 110

Asp Pro Asp Val Ser Arg Leu Thr Gln Asp Tyr Val Met Ile Phe Ile
115 120 125

Pro Gly Leu Pro Val Ile Phe Leu Tyr Asn Leu Leu Ala Lys Tyr Leu
130 135 140

Gln Asn Gln Val Gln Val Phe Glu Cys Val Gly Arg Pro Phe Ser Gln
145 150 155 160

His Thr Ala Leu Phe Gln Trp Glu Gly Gly Leu Gly Leu Ser Pro Ser
165 170 175

Leu His His Leu
180

<210> 93
<211> 44
<212> PRT
<213> Homo sapiens

<400> 93
Met Gly Val Ala Leu Pro Ser Pro Leu Leu Cys Ser Leu Pro Leu Phe
1 5 10 15

Leu Leu Phe Gly Asp Val Ser Gly Ser Ser Ser Leu Leu Ala Leu Leu
20 25 30

Pro Phe Leu His Pro Trp His His Pro Ser Leu Ser
35 40

<210> 94
<211> 403
<212> PRT
<213> Homo sapiens

<400> 94
Met Ala Thr Ala Glu Arg Arg Ala Leu Gly Ile Gly Phe Gln Trp Leu
1 5 10 15

Ser Leu Ala Thr Leu Val Leu Ile Cys Ala Gly Gln Gly Gly Arg Arg
20 25 30

Glu Asp Gly Gly Pro Ala Cys Tyr Gly Gly Phe Asp Leu Tyr Phe Ile
35 40 45

Leu Asp Lys Ser Gly Ser Val Leu His His Trp Asn Glu Ile Tyr Tyr
50 55 60

Phe Val Glu Gln Leu Ala His Lys Phe Ile Ser Pro Gln Leu Arg Met
65 70 75 80

Ser Phe Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr
85 90 95

Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu Glu Glu Leu Gln Lys Val
100 105 110

Leu Pro Gly Gly Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala Ser
115 120 125

Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val
130 135 140

Ile Ile Ala Leu Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe Tyr
145 150 155 160

Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Val Tyr
165 170 175

Cys Val Gly Val Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile Ala

180 185 190

Asp Ser Lys Asp His Val Phe Pro Val Asn Asp Gly Phe Gln Ala Leu
195 200 205

Gln Gly Ile Ile His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile Leu
210 215 220

Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val
225 230 235 240

Val Arg Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu
245 250 255

Cys Ser Phe Lys Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe
260 265 270

Ser Val Glu Asp Thr Tyr Leu Leu Cys Pro Ala Pro Ile Leu Lys Glu
275 280 285

Val Gly Met Lys Ala Ala Leu Gln Val Ser Met Asn Asp Gly Leu Ser
290 295 300

Phe Ile Ser Ser Ser Val Ile Ile Thr Thr Thr His Cys Ser Asp Gly
305 310 315 320

Ser Ile Leu Ala Ile Ala Leu Leu Ile Leu Phe Leu Leu Leu Ala Leu
325 330 335

Ala Leu Leu Trp Trp Phe Trp Pro Leu Cys Cys Thr Val Ile Ile Lys
340 345 350

Glu Val Pro Pro Pro Pro Ala Glu Glu Ser Glu Val Ser Asp His Ser
355 360 365

Arg Met Ala Val Gly Gly Gln Gly Gly Arg Val Gly Trp Arg Ala Gly
370 375 380

Trp Ala Ala Gly His Leu Ala Pro Cys Arg Ala Glu Leu Ser Gln Ala
385 390 395 400

Gln Arg Ile

<210> 95
<211> 870
<212> PRT
<213> Homo sapiens

<400> 95
Met Gly Pro Pro Ser Leu Val Leu Cys Leu Leu Ser Ala Thr Val Phe
1 5 10 15

Ser Leu Leu Gly Gly Ser Ser Ala Phe Leu Ser His His Arg Leu Lys
20 25 30

Gly Arg Phe Gln Arg Asp Arg Arg Asn Ile Arg Pro Asn Ile Ile Leu

```
        35                  40                  45

Val Leu Thr Asp Asp Gln Asp Val Glu Leu Gly Ser Met Gln Val Met
    50                  55                  60

Asn Lys Thr Arg Arg Ile Met Glu Gln Gly Gly Ala His Phe Ile Asn
65                  70                  75                  80

Ala Phe Val Thr Thr Pro Met Cys Cys Pro Ser Arg Ser Ser Ile Leu
                85                  90                  95

Thr Gly Lys Tyr Val His Asn His Asn Thr Tyr Thr Asn Asn Glu Asn
            100                 105                 110

Cys Ser Ser Pro Ser Trp Gln Ala Gln His Glu Ser Arg Thr Phe Ala
        115                 120                 125

Val Tyr Leu Asn Ser Thr Gly Tyr Arg Thr Ala Phe Phe Gly Lys Tyr
    130                 135                 140

Leu Asn Glu Tyr Asn Gly Ser Tyr Val Pro Pro Gly Trp Lys Glu Trp
145                 150                 155                 160

Val Gly Leu Leu Lys Asn Ser Arg Phe Tyr Asn Tyr Thr Leu Cys Arg
                165                 170                 175

Asn Gly Val Lys Glu Lys His Gly Ser Asp Tyr Ser Lys Asp Tyr Leu
            180                 185                 190

Thr Asp Leu Ile Thr Asn Asp Ser Val Ser Phe Phe Arg Thr Ser Lys
        195                 200                 205

Lys Met Tyr Pro His Arg Pro Val Leu Met Val Ile Ser His Ala Ala
    210                 215                 220

Pro His Gly Pro Glu Asp Ser Ala Pro Gln Tyr Ser Arg Leu Phe Pro
225                 230                 235                 240

Asn Ala Ser Gln His Ile Thr Pro Ser Tyr Asn Tyr Ala Pro Asn Pro
                245                 250                 255

Asp Lys His Trp Ile Met Arg Tyr Thr Gly Pro Met Lys Pro Ile His
            260                 265                 270

Met Glu Phe Thr Asn Met Leu Gln Arg Lys Arg Leu Gln Thr Leu Met
        275                 280                 285

Ser Val Asp Asp Ser Met Glu Thr Ile Tyr Asn Met Leu Val Glu Thr
    290                 295                 300

Gly Glu Leu Asp Asn Thr Tyr Ile Val Tyr Thr Ala Asp His Gly Tyr
305                 310                 315                 320

His Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser Met Pro Tyr Glu
                325                 330                 335

Phe Asp Ile Arg Val Pro Phe Tyr Val Arg Gly Pro Asn Val Glu Ala
            340                 345                 350
```

Gly Cys Leu Asn Pro His Ile Val Leu Asn Ile Asp Leu Ala Pro Thr
355 360 365

Ile Leu Asp Ile Ala Gly Leu Asp Ile Pro Ala Asp Met Asp Gly Lys
370 375 380

Ser Ile Leu Lys Leu Leu Asp Thr Glu Arg Pro Val Asn Arg Phe His
385 390 395 400

Leu Lys Lys Lys Met Arg Val Trp Arg Asp Ser Phe Leu Val Glu Arg
405 410 415

Gly Lys Leu Leu His Lys Arg Asp Asn Asp Lys Val Asp Ala Gln Glu
420 425 430

Glu Asn Phe Leu Pro Lys Tyr Gln Arg Val Lys Asp Leu Cys Gln Arg
435 440 445

Ala Glu Tyr Gln Thr Ala Cys Glu Gln Leu Gly Gln Lys Trp Gln Cys
450 455 460

Val Glu Asp Ala Thr Gly Lys Leu Lys Leu His Lys Cys Lys Gly Pro
465 470 475 480

Met Arg Leu Gly Gly Ser Arg Ala Leu Ser Asn Leu Val Pro Lys Tyr
485 490 495

Tyr Gly Gln Gly Ser Glu Ala Cys Thr Cys Asp Ser Gly Asp Tyr Lys
500 505 510

Leu Ser Leu Ala Gly Arg Arg Lys Lys Leu Phe Lys Lys Lys Tyr Lys
515 520 525

Ala Ser Tyr Val Arg Ser Arg Ser Ile Arg Ser Val Ala Ile Glu Val
530 535 540

Asp Gly Arg Val Tyr His Val Gly Leu Gly Asp Ala Ala Gln Pro Arg
545 550 555 560

Asn Leu Thr Lys Arg His Trp Pro Gly Ala Pro Glu Asp Gln Asp Asp
565 570 575

Lys Asp Gly Gly Asp Phe Ser Gly Thr Gly Gly Leu Pro Asp Tyr Ser
580 585 590

Ala Ala Asn Pro Ile Lys Val Thr His Arg Cys Tyr Ile Leu Glu Asn
595 600 605

Asp Thr Val Gln Cys Asp Leu Asp Leu Tyr Lys Ser Leu Gln Ala Trp
610 615 620

Lys Asp His Lys Leu His Ile Asp His Glu Ile Glu Thr Leu Gln Asn
625 630 635 640

Lys Ile Lys Asn Leu Arg Glu Val Arg Gly His Leu Lys Lys Lys Arg
645 650 655

Pro Glu Glu Cys Asp Cys His Lys Ile Ser Tyr His Thr Gln His Lys
660 665 670

Gly Arg Leu Lys His Arg Gly Ser Ser Leu His Pro Phe Arg Lys Gly
675 680 685

Leu Gln Glu Lys Asp Lys Val Trp Leu Leu Arg Glu Gln Lys Arg Lys
690 695 700

Lys Lys Leu Arg Lys Leu Leu Lys Arg Leu Gln Asn Asn Asp Thr Cys
705 710 715 720

Ser Met Pro Gly Leu Thr Cys Phe Thr His Asp Asn Gln His Trp Gln
725 730 735

Thr Ala Pro Phe Trp Thr Leu Gly Pro Phe Cys Ala Cys Thr Ser Ala
740 745 750

Asn Asn Asn Thr Tyr Trp Cys Met Arg Thr Ile Asn Glu Thr His Asn
755 760 765

Phe Leu Phe Cys Glu Phe Ala Thr Gly Phe Leu Glu Tyr Phe Asp Leu
770 775 780

Asn Thr Asp Pro Tyr Gln Leu Met Asn Ala Val Asn Thr Leu Asp Arg
785 790 795 800

Asp Val Leu Asn Gln Leu His Val Gln Leu Met Glu Leu Arg Ser Cys
805 810 815

Lys Gly Tyr Lys Gln Cys Asn Pro Arg Thr Arg Asn Met Asp Leu Gly
820 825 830

Leu Lys Asp Gly Gly Ser Tyr Glu Gln Tyr Arg Gln Phe Gln Arg Arg
835 840 845

Lys Trp Pro Glu Met Lys Arg Pro Ser Ser Lys Ser Leu Gly Gln Leu
850 855 860

Trp Glu Gly Trp Glu Gly
865 870

<210> 96
<211> 18
<212> PRT
<213> Homo sapiens

<400> 96
Met Val Arg Thr Leu Ser Leu Ala Val Leu Ser Trp Leu Pro Ala Ala
1 5 10 15

Val Cys

<210> 97
<211> 60

<212> PRT
<213> Homo sapiens

<400> 97
Met Leu Ser Ala Val Leu Thr Met Leu Arg Phe Ile Ile Ala Phe Ser
1 5 10 15

Leu Leu Phe Cys Ser Cys Ser Thr Asp Lys His Cys Thr Trp Tyr His
20 25 30

Ala Leu Pro His Phe Lys Lys Ile Cys Leu Thr Glu Arg Lys Lys Met
35 40 45

Trp Phe Gly Leu Ala Ala Val Leu Ile Tyr Gly Ile
50 55 60

<210> 98
<211> 318
<212> PRT
<213> Homo sapiens

<400> 98
Met Arg Leu Leu Ala Gly Trp Leu Cys Leu Ser Leu Ala Ser Val Trp
1 5 10 15

Leu Ala Arg Arg Met Trp Thr Leu Arg Ser Pro Leu Thr Arg Ser Leu
20 25 30

Tyr Val Asn Met Thr Ser Gly Pro Gly Gly Pro Ala Ala Ala Ala Gly
35 40 45

Gly Arg Lys Glu Asn His Gln Trp Tyr Val Cys Asn Arg Glu Lys Leu
50 55 60

Cys Glu Ser Leu Gln Ala Val Phe Val Gln Ser Tyr Leu Asp Gln Gly
65 70 75 80

Thr Gln Ile Phe Leu Asn Asn Ser Ile Glu Lys Ser Gly Trp Leu Phe
85 90 95

Ile Gln Leu Tyr His Ser Phe Val Ser Ser Val Phe Ser Leu Phe Met
100 105 110

Ser Arg Thr Ser Ile Asn Gly Leu Leu Gly Arg Gly Ser Met Phe Val
115 120 125

Phe Ser Pro Asp Gln Phe Gln Arg Leu Leu Lys Ile Asn Pro Asp Trp
130 135 140

Lys Thr His Arg Leu Leu Asp Leu Gly Ala Gly Asp Gly Glu Val Thr
145 150 155 160

Lys Ile Met Ser Pro His Phe Glu Glu Ile Tyr Ala Thr Glu Leu Ser
165 170 175

Glu Thr Met Ile Trp Gln Leu Gln Lys Lys Lys Tyr Arg Val Leu Gly
180 185 190

Ile Asn Glu Trp Gln Asn Thr Gly Phe Gln Tyr Asp Val Ile Ser Cys
        195   200   205

Leu Asn Leu Leu Asp Arg Cys Asp Gln Pro Leu Thr Leu Leu Lys Asp
    210   215   220

Ile Arg Ser Val Leu Glu Pro Thr Arg Gly Arg Val Ile Leu Ala Leu
225   230   235   240

Val Leu Pro Phe His Pro Tyr Val Glu Asn Val Gly Gly Lys Trp Glu
                245   250   255

Lys Pro Ser Glu Ile Leu Glu Ile Lys Gly Gln Asn Trp Glu Glu Gln
            260   265   270

Val Asn Ser Leu Pro Glu Val Phe Arg Lys Ala Gly Phe Val Ile Glu
        275   280   285

Ala Phe Thr Arg Leu Pro Tyr Leu Cys Glu Gly Asp Met Tyr Asn Ala
    290   295   300

Tyr Tyr Val Leu Asp Asp Ala Val Phe Val Leu Lys Pro Val
305   310   315

<210> 99
<211> 35
<212> PRT
<213> Homo sapiens

<400> 99
Met Ser Leu Gly Phe Trp Val Trp Leu Pro Ser Cys Cys His Lys Met
  1   5   10   15

Leu Val Val Thr Cys Thr Phe Gly His Tyr Leu Pro Leu Glu Ser Ser
            20   25   30

His His Leu
        35

<210> 100
<211> 81
<212> PRT
<213> Homo sapiens

<400> 100
Met Ile Leu Gly Ile His Trp Gly Ile Phe Leu Leu Leu Leu Leu Ser
  1   5   10   15

Trp Leu Glu Leu Gln Arg Thr Val Ile Phe Phe Phe Ser Pro Phe Pro
            20   25   30

Ile Gln Lys His Tyr Thr Leu Gly His Phe Ser Phe Ser Gln Arg Arg
        35   40   45

Phe Met Asp Ser Gln Thr Glu Leu Cys Ala Thr Gly Lys Val Lys Arg

50 55 60

Glu Lys Ala Ala Asp Glu Val Thr Trp Leu His Val Leu His His Ala
65 70 75 80

Glu

<210> 101
<211> 42
<212> PRT
<213> Homo sapiens

<400> 101
Met Phe Lys Lys Asp Leu Ile Cys Lys Arg Trp Ser Phe Phe Phe Trp
1 5 10 15

Gly Leu Leu Ile Ser Val Val Ile Leu Thr Ser Phe Ser Asn Tyr Ser
20 25 30

Arg Arg Phe Tyr Leu Asp Leu Tyr Phe Ser
35 40

<210> 102
<211> 49
<212> PRT
<213> Homo sapiens

<400> 102
Met Ser Pro Arg Pro Leu Ile Ala Arg Cys Glu Ala Leu Gly Cys Gly
1 5 10 15

Ala Arg Arg Leu Pro Trp Trp Ala Leu Ala Met Ala Leu Cys Ala Cys
20 25 30

Gly Arg Cys Val Ala Ala Asn Ser Ile Gly Glu Thr Leu Pro Ser Glu
35 40 45

Val

<210> 103
<211> 54
<212> PRT
<213> Homo sapiens

<400> 103
Met Val Gly Leu Pro Ala Val Val Gln Leu Phe Trp Gly Leu Cys Leu
1 5 10 15

Cys Thr Cys Gly Ala Val Ser Cys Pro Thr Glu Leu Ala Val Gln Trp
20 25 30

Arg Ile Gln Ser Asp Ile Trp Cys Ser Leu Arg Lys Asn Val Ala Pro
35 40 45

Glu Ala Cys Gln Trp Leu
50

<210> 104
<211> 44
<212> PRT
<213> Homo sapiens

<400> 104
Met Ala Pro Ala Cys Gln Ile Leu Arg Trp Ala Leu Ala Leu Gly Leu
1 5 10 15

Gly Leu Met Phe Glu Val Thr His Ala Phe Arg Ser Gln Gly Arg Gly
20 25 30

Ser Leu Val Val Ala Val Gly Arg Glu Arg Lys Met
35 40

<210> 105
<211> 24
<212> PRT
<213> Homo sapiens

<400> 105
Met Trp His Leu Trp Arg Arg Leu Leu Ser Cys Phe Pro Val Ala Met
1 5 10 15

Leu Gln Asp Tyr Lys Tyr Ser Val
20

<210> 106
<211> 37
<212> PRT
<213> Homo sapiens

<400> 106
Met Val Glu Ser Pro Val Cys Gly Leu Leu Glu Gly Trp Phe Phe Leu
1 5 10 15

Leu Phe Ser Leu Ala Phe Leu Ser Thr His Leu Phe Ser Glu Ala Ser
20 25 30

Pro Leu Ser Ile Leu
35

<210> 107
<211> 58
<212> PRT
<213> Homo sapiens

<400> 107
Met Thr Leu Ser Val Leu Gln His Phe Phe Ile Cys Val Leu Leu Ile
1 5 10 15

Leu Leu Leu Asp Thr Asn Leu Cys Arg Gln Ile Ser Ser His Ser Phe
20 25 30

Glu Phe Ser Gly Asn Gln Pro Leu Val Phe Cys Cys Ile Ser Ser Ile
35 40 45

Ser Ala Lys Leu Val Leu Asp Gln Ala Gly
50 55

<210> 108
<211> 283
<212> PRT
<213> Homo sapiens

<400> 108
Met Lys Ile Val Pro Leu Thr Ala Ala Val Leu Ala Leu Val Leu Ala
1 5 10 15

Pro Ala Ala His Ala Gln Pro Ala Asn Lys Ala Thr Thr Val Ser Pro
20 25 30

Thr Ala Ala Ala Phe Leu Ala Gln Phe Ala Thr Glu Gly Asn Asp Ser
35 40 45

Val Ser Trp Ala Gln Phe Glu Ala Phe Arg Lys Gln Arg Tyr Ala Asp
50 55 60

Thr Asp Arg Asn Gln Asp Gly His Val Asp Glu Gln Glu Tyr Val Asp
65 70 75 80

Glu Tyr Leu Gln Arg Phe Asp Val Arg Leu Ala Asp Ala Arg Ala Gly
85 90 95

His Leu Arg Gln Thr Asp Thr Arg Phe Lys Ala Leu Asp Arg Asp Gly
100 105 110

Asn Gly Ala Ile Ser Arg Ala Glu Tyr Asp Ala Ala Gly Glu Arg Thr
115 120 125

Trp Ala Gly Tyr Glu Arg Ser Gln Asn Ala Thr Gln Glu Thr Ala Ala
130 135 140

Ala Ser Ser Arg Asp Pro Leu Lys Met Pro Thr Ser His Thr Ala Asn
145 150 155 160

Gly Met Leu Asp Leu Tyr Asp Arg Asn Lys Asp Gly Ala Val Asp Arg
165 170 175

Glu Glu Phe Asp Ala Val Arg Ala Ala Ser Phe Ala Ala Thr Asp Thr
180 185 190

Asp Gly Asn Gly Thr Leu Ser Leu Ala Glu Tyr Thr Ala Glu Phe Glu
195 200 205

Gly Arg Leu Asp Gln Gln Arg Gln Arg Val Arg Ala Asp Ala Glu Arg
210 215 220

Gln Ala Arg Val Arg Phe Ala Ser Leu Asp Lys Asp Thr Asp Gly Arg
225 230 235 240

Met Thr Phe Ala Glu Tyr Gln Leu Ser Gly Lys Arg Met Phe Asp Arg
245 250 255

Ala Asp Ser Asn Gly Asp Gly Val Val Asp Ala Arg Asp Pro Glu Pro
260 265 270

Val Ala Gly Ala His Ser Ala Asn Gly Asn Arg
275 280

<210> 109
<211> 42
<212> PRT
<213> Homo sapiens

<400> 109
Met Leu Leu Ser Trp Thr Val Leu Ile Ile Ile Leu Pro Phe Ala Gly
1 5 10 15

Asp Val Ser Ser His Leu Cys Ile Leu Arg Pro Phe Ala Gly Ser Val
20 25 30

Ser Ser Cys Leu Ser Asn Phe Lys Arg Ile
35 40

<210> 110
<211> 29
<212> PRT
<213> Homo sapiens

<400> 110
Met Leu Cys Ser Asn Ser Phe Ser Pro Ser Leu Ser Val Tyr Leu Cys
1 5 10 15

Ser Leu Cys Phe Ser Leu Val Ser Ser Lys Ser Ser Lys
20 25

<210> 111
<211> 737
<212> PRT
<213> Homo sapiens

<400> 111
Met Glu Val Arg Lys Leu Ser Ile Ser Trp Gln Phe Leu Ile Val Leu
1 5 10 15

Val Leu Ile Leu Gln Ile Leu Ser Ala Leu Asp Phe Asp Pro Tyr Arg
20 25 30

Val Leu Gly Val Ser Arg Thr Ala Ser Gln Ala Asp Ile Lys Lys Ala
35 40 45

Tyr Lys Lys Leu Ala Arg Glu Trp His Pro Asp Lys Asn Lys Asp Pro
50 55 60

Gly Ala Glu Asp Lys Phe Ile Gln Ile Ser Lys Ala Tyr Glu Ile Leu
65 70 75 80

Ser Asn Glu Glu Lys Arg Ser Asn Tyr Asp Gln Tyr Gly Asp Ala Gly
85 90 95

Glu Asn Gln Gly Tyr Gln Lys Gln Gln Gln Gln Arg Glu Tyr Arg Phe
100 105 110

Arg His Phe His Glu Asn Phe Tyr Phe Asp Glu Ser Phe Phe His Phe
115 120 125

Pro Phe Asn Ser Glu Arg Arg Asp Ser Ile Asp Glu Lys Tyr Leu Leu
130 135 140

His Phe Ser His Tyr Val Asn Glu Val Val Pro Asp Ser Phe Lys Lys
145 150 155 160

Pro Tyr Leu Ile Lys Ile Thr Ser Asp Trp Cys Phe Ser Cys Ile His
165 170 175

Ile Glu Pro Val Trp Lys Glu Val Ile Gln Glu Leu Glu Glu Leu Gly
180 185 190

Val Gly Ile Gly Val Val His Ala Gly Tyr Glu Arg Arg Leu Ala His
195 200 205

His Leu Gly Ala His Ser Thr Pro Ser Ile Leu Gly Ile Ile Asn Gly
210 215 220

Lys Ile Ser Phe Phe His Asn Ala Val Val Arg Glu Asn Leu Arg Gln
225 230 235 240

Phe Val Glu Ser Leu Leu Pro Gly Asn Leu Val Glu Lys Val Thr Asn
245 250 255

Lys Asn Tyr Val Arg Phe Leu Ser Gly Trp Gln Gln Glu Asn Lys Pro
260 265 270

His Val Leu Leu Phe Asp Gln Thr Pro Ile Val Pro Leu Leu Tyr Lys
275 280 285

Leu Thr Ala Phe Ala Tyr Lys Asp Tyr Leu Ser Phe Gly Tyr Val Tyr
290 295 300

Val Gly Leu Arg Gly Thr Glu Glu Met Thr Arg Arg Tyr Asn Ile Asn
305 310 315 320

Ile Tyr Ala Pro Thr Leu Leu Val Phe Lys Glu His Ile Asn Arg Pro
325 330 335

Ala Asp Val Ile Gln Ala Arg Gly Met Lys Lys Gln Ile Ile Asp Asp
340 345 350

Phe Ile Thr Arg Asn Lys Tyr Leu Leu Ala Ala Arg Leu Thr Ser Gln

355 360 365

Lys Leu Phe His Glu Leu Cys Pro Val Lys Arg Ser His Arg Gln Arg
370 375 380

Lys Tyr Cys Val Val Leu Leu Thr Ala Glu Thr Thr Lys Leu Ser Lys
385 390 395 400

Pro Phe Glu Ala Phe Leu Ser Phe Ala Leu Ala Asn Thr Gln Asp Thr
405 410 415

Val Arg Phe Val His Val Tyr Ser Asn Arg Gln Gln Glu Phe Ala Asp
420 425 430

Thr Leu Leu Pro Asp Ser Glu Ala Phe Gln Gly Lys Ser Ala Val Ser
435 440 445

Ile Leu Glu Arg Arg Asn Thr Ala Gly Arg Val Val Tyr Lys Thr Leu
450 455 460

Glu Asp Pro Trp Ile Gly Ser Glu Ser Asp Lys Phe Ile Leu Leu Gly
465 470 475 480

Tyr Leu Asp Gln Leu Arg Lys Asp Pro Ala Leu Leu Ser Ser Glu Ala
485 490 495

Val Leu Pro Asp Leu Thr Asp Glu Leu Ala Pro Val Phe Leu Leu Arg
500 505 510

Trp Phe Tyr Ser Ala Ser Asp Tyr Ile Ser Asp Cys Trp Asp Ser Ile
515 520 525

Phe His Asn Asn Trp Arg Glu Met Met Pro Leu Leu Ser Leu Ile Phe
530 535 540

Ser Ala Leu Phe Ile Leu Phe Gly Thr Val Ile Val Gln Ala Phe Ser
545 550 555 560

Asp Ser Asn Asp Glu Arg Glu Ser Ser Pro Pro Glu Lys Glu Glu Ala
565 570 575

Gln Glu Lys Thr Gly Lys Thr Glu Pro Ser Phe Thr Lys Glu Asn Ser
580 585 590

Ser Lys Ile Pro Lys Lys Gly Phe Val Glu Val Thr Glu Leu Thr Asp
595 600 605

Val Thr Tyr Thr Ser Asn Leu Val Arg Leu Arg Pro Gly His Met Asn
610 615 620

Val Val Leu Ile Leu Ser Asn Ser Thr Lys Thr Ser Leu Leu Gln Lys
625 630 635 640

Phe Ala Leu Glu Val Tyr Thr Phe Thr Gly Ser Ser Cys Leu His Phe
645 650 655

Ser Phe Leu Ser Leu Asp Lys His Arg Glu Trp Leu Glu Tyr Leu Leu
660 665 670

Glu Phe Ala Gln Asp Ala Ala Pro Ile Pro Asn Gln Tyr Asp Lys His
675 680 685

Phe Met Glu Arg Asp Tyr Thr Gly Tyr Val Leu Ala Leu Asn Gly His
690 695 700

Lys Lys Tyr Phe Cys Leu Phe Lys Pro Gln Lys Thr Val Glu Glu Glu
705 710 715 720

Glu Ala Ile Gly Ser Cys Ser Asp Val Asp Ser Ser Leu Tyr Leu Gly
725 730 735

Glu

<210> 112
<211> 42
<212> PRT
<213> Homo sapiens

<400> 112
Met Asn Ser Ser Phe Phe Ile Ser Leu Pro Ala Leu Ile Trp Ser Val
1 5 10 15

Cys Leu Ile Leu Gly Trp Trp Gln Val Ser Ser Gly Lys Val Ala His
20 25 30

Cys Gly Phe Ile Phe Cys Phe Pro Asn Asn
35 40

<210> 113
<211> 128
<212> PRT
<213> Homo sapiens

<400> 113
Met Pro Val Phe Val Cys Ser Ile Gly Leu Cys Phe Leu Phe Ser Ile
1 5 10 15

Leu Leu Leu Phe Pro Pro Phe Gln Phe Ser Tyr Ile Cys Trp Leu Ser
20 25 30

Gln Ala Ser Val Tyr Ser Pro Ser Pro Ser Leu Ser Asn Leu Glu Val
35 40 45

Leu Leu Cys Leu Ser Ile Leu Leu Met Ile Ile Phe Pro Phe Leu Ile
50 55 60

Ser Ile Ile His Ile Phe Ser Ile Gly Arg Leu Ser Thr His Met Gly
65 70 75 80

Ala His Thr His Thr His Thr His Thr His Thr His Thr His Thr Gln
85 90 95

Val Cys Tyr Trp Pro Leu Leu Leu Ile Ser Gln Glu Asn Glu Pro Phe

100 105 110

Arg Met Phe Leu Pro Leu His Ser Ala Leu Thr Gln Asn Phe Cys Ser
115 120 125

<210> 114
<211> 57
<212> PRT
<213> Homo sapiens

<400> 114
Met Pro Pro His Arg Gln Thr Asp Gly Gln Met Gly Leu Pro Ala Pro
1 5 10 15

Ala Leu Trp Val Trp Gly Leu Leu Leu Ser Ser Ser Phe Gln Thr Leu
20 25 30

Leu Pro Ala Phe Pro Lys Pro Pro Ala Leu Asn Leu Gly Cys Ser Thr
35 40 45

Arg Pro Ile Pro Ser Phe Leu Lys Ile
50 55

<210> 115
<211> 38
<212> PRT
<213> Homo sapiens

<400> 115
Met Ser Trp Arg Val Trp Ala Leu Leu Phe Phe Pro Ala Val Cys Val
1 5 10 15

Cys Val Cys Val Cys Val Cys Ala Cys Thr Arg Thr Arg Val Cys Asp
20 25 30

Glu Thr Ile Lys Leu Val
35

<210> 116
<211> 57
<212> PRT
<213> Homo sapiens

<400> 116
Met Gly Leu Leu Pro Gly Trp Leu Leu Leu Trp Ala Arg Leu Lys Cys
1 5 10 15

Phe Cys Ala Val Gly Leu Gly Ser Leu Ala Ala Val Tyr Gly Arg Gly
20 25 30

Pro Gly Leu Pro Gln Asp Gln Leu Asp Cys Val Leu Trp Asp Cys Gly
35 40 45

Thr Leu Gly Leu Tyr Arg Gly Gln Phe
50 55

<210> 117
<211> 48
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (31)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 117
Met Gly Glu Thr Leu Val Ser Val Phe Leu Lys Pro Pro Ala Leu Thr
1 5 10 15

Trp Leu Leu Arg Ala Ile Cys Leu Met Val Gln Thr Trp Ala Xaa Gly
20 25 30

Gln Arg Ser Trp Pro Gln Ser Leu Ala Leu Pro Cys Tyr Leu Asn Arg
35 40 45

<210> 118
<211> 25
<212> PRT
<213> Homo sapiens

<400> 118
Met Val Gly Arg Cys Ser Ile Leu Ser Ser Thr Pro Gln Arg His Pro
1 5 10 15

Ser Leu Ser Trp Glu Gly Leu Gly Gly
20 25

<210> 119
<211> 439
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (358)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 119
Met Val Pro Ser Ser Pro Arg Ala Leu Phe Leu Leu Leu Leu Ile Leu
1 5 10 15

Ala Cys Pro Glu Pro Arg Ala Ser Gln Asn Cys Leu Ser Lys Gln Gln
20 25 30

Leu Leu Ser Ala Ile Arg Gln Leu Gln Gln Leu Leu Lys Gly Gln Glu
35 40 45

Thr Arg Phe Ala Glu Gly Ile Arg His Met Lys Ser Arg Leu Ala Ala
50 55 60

Leu Gln Asn Ser Val Gly Arg Val Gly Pro Asp Ala Leu Pro Val Ser
65 70 75 80

Cys Pro Ala Leu Asn Thr Pro Ala Asp Gly Arg Lys Phe Gly Ser Lys
85 90 95

Tyr Leu Val Asp His Glu Val His Phe Thr Cys Asn Pro Gly Phe Arg
100 105 110

Leu Val Gly Pro Ser Ser Val Val Cys Leu Pro Asn Gly Thr Trp Thr
115 120 125

Gly Glu Gln Pro His Cys Arg Gly Ile Ser Glu Cys Ser Ser Gln Pro
130 135 140

Cys Gln Asn Gly Gly Thr Cys Val Glu Gly Val Asn Gln Tyr Arg Cys
145 150 155 160

Ile Cys Pro Pro Gly Arg Thr Gly Asn Arg Cys Gln His Gln Ala Gln
165 170 175

Thr Ala Ala Pro Glu Gly Ser Val Ala Gly Asp Ser Ala Phe Ser Arg
180 185 190

Ala Pro Arg Cys Ala Gln Val Glu Arg Ala Gln His Cys Ser Cys Glu
195 200 205

Ala Gly Phe His Leu Ser Gly Ala Ala Gly Asp Ser Val Cys Gln Asp
210 215 220

Val Asn Glu Cys Glu Leu Tyr Gly Gln Glu Gly Arg Pro Arg Leu Cys
225 230 235 240

Met His Ala Cys Val Asn Thr Pro Gly Ser Tyr Arg Cys Thr Cys Pro
245 250 255

Gly Gly Tyr Arg Thr Leu Ala Asp Gly Lys Ser Cys Glu Asp Val Asp
260 265 270

Glu Cys Val Gly Leu Gln Pro Val Cys Pro Gln Gly Thr Thr Cys Ile
275 280 285

Asn Thr Gly Gly Ser Phe Gln Cys Val Ser Pro Glu Cys Pro Glu Gly
290 295 300

Ser Gly Asn Val Ser Tyr Val Lys Thr Ser Pro Phe Gln Cys Glu Arg
305 310 315 320

Asn Pro Cys Pro Met Asp Ser Arg Pro Cys Arg His Leu Pro Lys Thr
325 330 335

Ile Ser Phe His Tyr Leu Ser Leu Pro Ser Asn Leu Lys Thr Pro Ile
340 345 350

Thr Leu Phe Arg Met Xaa Thr Ala Ser Ala Pro Gly Arg Ala Gly Pro
355 360 365

Asn Ser Leu Arg Phe Gly Ile Val Gly Gly Asn Ser Arg Gly His Phe
370 375 380

Val Met Gln Arg Ser Asp Arg Gln Thr Gly Asp Leu Ile Leu Val Gln
385 390 395 400

Asn Leu Glu Gly Pro Gln Thr Leu Glu Val Asp Val Asp Met Ser Glu
405 410 415

Tyr Leu Asp Arg Ser Phe Gln Ala Asn His Val Ser Lys Val Thr Ile
420 425 430

Phe Val Ser Pro Tyr Asp Phe
435

<210> 120
<211> 195
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (159)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (175)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 120
Met Trp Thr Leu Phe Ala Leu Ser Gly Pro Leu Phe Leu Phe Gln Val
1 5 10 15

Leu Thr Phe Met Ile Tyr Ile Val Ser Thr Val Phe Cys Gly His Leu
20 25 30

Gly Lys Val Glu Leu Ala Ser Val Thr Leu Ala Val Ala Phe Val Asn
35 40 45

Val Cys Gly Val Ser Val Gly Val Gly Leu Ser Ser Ala Cys Asp Thr
50 55 60

Leu Met Ser Gln Ser Phe Gly Ser Pro Asn Lys Lys His Val Gly Val
65 70 75 80

Ile Leu Gln Arg Gly Ala Leu Val Leu Leu Leu Cys Cys Leu Pro Cys
85 90 95

Trp Ala Leu Phe Leu Asn Thr Gln His Ile Leu Leu Leu Phe Arg Gln
100 105 110

Asp Pro Asp Val Ser Arg Leu Thr Gln Asp Tyr Val Met Ile Phe Ile
115 120 125

Pro Gly Leu Pro Val Ile Phe Leu Tyr Asn Leu Leu Ala Lys Tyr Leu
130 135 140

Gln Asn Gln Val Gln Val Phe Ser Val Trp Gly Gly Pro Ser Xaa Ser
145 150 155 160

Thr Leu Pro Tyr Ser Ser Gly Arg Gly Ala Trp Gly Phe Pro Xaa Leu
165 170 175

Ser Thr Ile Cys Glu Pro Ala Leu Glu Arg Gly Ser Leu Pro Thr His
180 185 190

Leu Pro Tyr
195

<210> 121
<211> 3
<212> PRT
<213> Homo sapiens

<400> 121
Ile Pro Gly
1

<210> 122
<211> 37
<212> PRT
<213> Homo sapiens

<400> 122
Leu Ala Gly Pro Val Phe Ile Tyr Phe Arg Arg Ser Pro Gly Pro Lys
1 5 10 15

Ser Ser Val Val Trp Trp Ala Thr Val Ser Thr Val Trp Pro Thr Met
20 25 30

Pro Trp Phe Leu Cys
35

<210> 123
<211> 44
<212> PRT
<213> Homo sapiens

<400> 123
Met Gly Val Ala Leu Pro Ser Pro Leu Leu Cys Ser Leu Pro Leu Phe
1 5 10 15

Leu Leu Phe Gly Asp Val Ser Gly Ser Ser Ser Leu Leu Ala Leu Leu
20 25 30

Pro Phe Leu His Pro Trp His His Pro Ser Leu Ser
35 40

<210> 124
<211> 86
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (6)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (21)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (31)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (76)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 124
Leu Gly Ser Pro Glu Xaa Ala Gln Lys Val Asp Ile Thr Ser Ala His
1 5 10 15

Phe Ile Gly Gln Xaa Ser Arg Pro Ser Asp Phe Ala Gln Val Xaa Ser
20 25 30

Leu Glu Gly Ser Arg Pro Val Ile Trp Ser Leu Asn Gly Trp Thr Leu
35 40 45

Lys Glu Thr Pro Arg Ala Asp Gly Val Phe Thr Glu Thr Ala Gly Gln
50 55 60

Gly Leu Gly Thr Ala Gln Gly His Leu Leu Trp Xaa Ala Ala Ala Thr
65 70 75 80

Gly Ser Pro Asp Cys Ser
85

<210> 125
<211> 403
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (175)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (320)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (331)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (368)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 125
Met Ala Thr Ala Glu Arg Arg Ala Leu Gly Ile Gly Phe Gln Trp Leu
1 5 10 15

Ser Leu Ala Thr Leu Val Leu Ile Cys Ala Gly Gln Gly Gly Arg Arg
20 25 30

Glu Asp Gly Gly Pro Ala Cys Tyr Gly Gly Phe Asp Leu Tyr Phe Ile
35 40 45

Leu Asp Lys Ser Gly Ser Val Leu His His Trp Asn Glu Ile Tyr Tyr
50 55 60

Phe Val Glu Gln Leu Ala His Lys Phe Ile Ser Pro Gln Leu Arg Met
65 70 75 80

Ser Phe Ile Val Phe Ser Thr Arg Gly Thr Thr Leu Met Lys Leu Thr
85 90 95

Glu Asp Arg Glu Gln Ile Arg Gln Gly Leu Glu Glu Leu Gln Lys Val
100 105 110

Leu Pro Gly Gly Asp Thr Tyr Met His Glu Gly Phe Glu Arg Ala Ser
115 120 125

Glu Gln Ile Tyr Tyr Glu Asn Arg Gln Gly Tyr Arg Thr Ala Ser Val
130 135 140

Ile Ile Ala Leu Thr Asp Gly Glu Leu His Glu Asp Leu Phe Phe Tyr
145 150 155 160

Ser Glu Arg Glu Ala Asn Arg Ser Arg Asp Leu Gly Ala Ile Xaa Tyr
165 170 175

Cys Val Gly Val Lys Asp Phe Asn Glu Thr Gln Leu Ala Arg Ile Ala
180 185 190

Asp Ser Lys Asp His Val Phe Pro Val Asn Asp Gly Phe Gln Ala Leu
195 200 205

Gln Gly Ile Ile His Ser Ile Leu Lys Lys Ser Cys Ile Glu Ile Leu
210 215 220

Ala Ala Glu Pro Ser Thr Ile Cys Ala Gly Glu Ser Phe Gln Val Val
225 230 235 240

Val Arg Gly Asn Gly Phe Arg His Ala Arg Asn Val Asp Arg Val Leu
245 250 255

Cys Ser Phe Lys Ile Asn Asp Ser Val Thr Leu Asn Glu Lys Pro Phe
260 265 270

Ser Val Glu Asp Thr Tyr Leu Leu Cys Pro Ala Pro Ile Leu Lys Glu
275 280 285

Val Gly Met Lys Ala Ala Leu Gln Val Ser Met Asn Asp Gly Leu Ser
290 295 300

Phe Ile Ser Ser Ser Val Ile Ile Thr Thr Thr His Cys Ser Asp Xaa
305 310 315 320

Ser Ile Leu Ala Ile Ala Leu Leu Ile Leu Xaa Leu Leu Leu Ala Leu
325 330 335

Ala Leu Leu Trp Trp Phe Trp Pro Leu Cys Cys Thr Val Ile Ile Lys
340 345 350

Glu Val Pro Pro Pro Pro Ala Glu Glu Ser Glu Val Ser Asp His Xaa
355 360 365

Arg Met Ala Val Gly Gly Gln Gly Gly Arg Val Gly Trp Arg Ala Gly
370 375 380

Trp Ala Ala Gly His Leu Ala Pro Cys Arg Ala Glu Leu Ser Gln Ala
385 390 395 400

Gln Arg Ile

<210> 126
<211> 93
<212> PRT
<213> Homo sapiens

<400> 126
Ser Ala Ser Cys Trp Asn Ala Asn Phe Leu Pro Arg Asn Gln Gly Arg
1 5 10 15

Lys Leu His Cys Cys Ala Lys Lys Lys Lys Lys Pro Ser Leu His Thr
20 25 30

Leu Lys Pro Phe Leu Asn Pro Ser Arg Glu Ser Thr Val Ala Ser Ser
35 40 45

Thr Thr Ala Ile Gly Phe Ala Ser Val Met Cys Ser Tyr Leu Leu Asp
50 55 60

Phe Gln Asn Ile Lys Lys Lys Lys Arg Ala Ala Ala Leu Glu Asp Pro
65 70 75 80

```
Ser Leu Arg Thr Arg Ala Cys Asp Asn Ile Ala Arg Arg
                85                  90


<210> 127
<211> 870
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (534)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 127
Met Gly Pro Pro Ser Leu Val Leu Cys Leu Leu Ser Ala Thr Val Phe
1               5                   10                  15

Ser Leu Leu Gly Gly Ser Ser Ala Phe Leu Ser His His Arg Leu Lys
            20                  25                  30

Gly Arg Phe Gln Arg Asp Arg Arg Asn Ile Arg Pro Asn Ile Ile Leu
        35                  40                  45

Val Leu Thr Asp Asp Gln Asp Val Glu Leu Gly Ser Met Gln Val Met
    50                  55                  60

Asn Lys Thr Arg Arg Ile Met Glu Gln Gly Gly Ala His Phe Ile Asn
65                  70                  75                  80

Ala Phe Val Thr Thr Pro Met Cys Cys Pro Ser Arg Ser Ser Ile Leu
                85                  90                  95

Thr Gly Lys Tyr Val His Asn His Asn Thr Tyr Thr Asn Asn Glu Asn
            100                 105                 110

Cys Ser Ser Pro Ser Trp Gln Ala Gln His Glu Ser Arg Thr Phe Ala
        115                 120                 125

Val Tyr Leu Asn Ser Thr Gly Tyr Arg Thr Ala Phe Phe Gly Lys Tyr
    130                 135                 140

Leu Asn Glu Tyr Asn Gly Ser Tyr Val Pro Pro Gly Trp Lys Glu Trp
145                 150                 155                 160

Val Gly Leu Leu Lys Asn Ser Arg Phe Tyr Asn Tyr Thr Leu Cys Arg
                165                 170                 175

Asn Gly Val Lys Glu Lys His Gly Ser Asp Tyr Ser Lys Asp Tyr Leu
            180                 185                 190

Thr Asp Leu Ile Thr Asn Asp Ser Val Ser Phe Phe Arg Thr Ser Lys
        195                 200                 205

Lys Met Tyr Pro His Arg Pro Val Leu Met Val Ile Ser His Ala Ala
    210                 215                 220
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Pro His Gly Pro Glu Asp Ser Ala Pro Gln Tyr Ser Arg Leu Phe Pro
225 230 235 240

Asn Ala Ser Gln His Ile Thr Pro Ser Tyr Asn Tyr Ala Pro Asn Pro
245 250 255

Asp Lys His Trp Ile Met Arg Tyr Thr Gly Pro Met Lys Pro Ile His
260 265 270

Met Glu Phe Thr Asn Met Leu Gln Arg Lys Arg Leu Gln Thr Leu Met
275 280 285

Ser Val Asp Asp Ser Met Glu Thr Ile Tyr Asn Met Leu Val Glu Thr
290 295 300

Gly Glu Leu Asp Asn Thr Tyr Ile Val Tyr Thr Ala Asp His Gly Tyr
305 310 315 320

His Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser Met Pro Tyr Glu
325 330 335

Phe Asp Ile Arg Val Pro Phe Tyr Val Arg Gly Pro Asn Val Glu Ala
340 345 350

Gly Cys Leu Asn Pro His Ile Val Leu Asn Ile Asp Leu Ala Pro Thr
355 360 365

Ile Leu Asp Ile Ala Gly Leu Asp Ile Pro Ala Asp Met Asp Gly Lys
370 375 380

Ser Ile Leu Lys Leu Leu Asp Thr Glu Arg Pro Val Asn Arg Phe His
385 390 395 400

Leu Lys Lys Lys Met Arg Val Trp Arg Asp Ser Phe Leu Val Glu Arg
405 410 415

Gly Lys Leu Leu His Lys Arg Asp Asn Asp Lys Val Asp Ala Gln Glu
420 425 430

Glu Asn Phe Leu Pro Lys Tyr Gln Arg Val Lys Asp Leu Cys Gln Arg
435 440 445

Ala Glu Tyr Gln Thr Ala Cys Glu Gln Leu Gly Gln Lys Trp Gln Cys
450 455 460

Val Glu Asp Ala Thr Gly Lys Leu Lys Leu His Lys Cys Lys Gly Pro
465 470 475 480

Met Arg Leu Gly Gly Ser Arg Ala Leu Ser Asn Leu Val Pro Lys Tyr
485 490 495

Tyr Gly Gln Gly Ser Glu Ala Cys Thr Cys Asp Ser Gly Asp Tyr Lys
500 505 510

Leu Ser Leu Ala Gly Arg Arg Lys Lys Leu Phe Lys Lys Lys Tyr Lys
515 520 525

Ala Ser Tyr Val Arg Xaa Arg Ser Ile Arg Ser Val Ala Ile Glu Val

530 535 540

Asp Gly Arg Val Tyr His Val Gly Leu Gly Asp Ala Ala Gln Pro Arg
545 550 555 560

Asn Leu Thr Lys Arg His Trp Pro Gly Ala Pro Glu Asp Gln Asp Asp
565 570 575

Lys Asp Gly Gly Asp Phe Ser Gly Thr Gly Gly Leu Pro Asp Tyr Ser
580 585 590

Ala Ala Asn Pro Ile Lys Val Thr His Arg Cys Tyr Ile Leu Glu Asn
595 600 605

Asp Thr Val Gln Cys Asp Leu Asp Leu Tyr Lys Ser Leu Gln Ala Trp
610 615 620

Lys Asp His Lys Leu His Ile Asp His Glu Ile Glu Thr Leu Gln Asn
625 630 635 640

Lys Ile Lys Asn Leu Arg Glu Val Arg Gly His Leu Lys Lys Lys Arg
645 650 655

Pro Glu Glu Cys Asp Cys His Lys Ile Ser Tyr His Thr Gln His Lys
660 665 670

Gly Arg Leu Lys His Arg Gly Ser Ser Leu His Pro Phe Arg Lys Gly
675 680 685

Leu Gln Glu Lys Asp Lys Val Trp Leu Leu Arg Glu Gln Lys Arg Lys
690 695 700

Lys Lys Leu Arg Lys Leu Leu Lys Arg Leu Gln Asn Asn Asp Thr Cys
705 710 715 720

Ser Met Pro Gly Leu Thr Cys Phe Thr His Asp Asn Gln His Trp Gln
725 730 735

Thr Ala Pro Phe Trp Thr Leu Gly Pro Phe Cys Ala Cys Thr Ser Ala
740 745 750

Asn Asn Asn Thr Tyr Trp Cys Met Arg Thr Ile Asn Glu Thr His Asn
755 760 765

Phe Leu Phe Cys Glu Phe Ala Thr Gly Phe Leu Glu Tyr Phe Asp Leu
770 775 780

Asn Thr Asp Pro Tyr Gln Leu Met Asn Ala Val Asn Thr Leu Asp Arg
785 790 795 800

Asp Val Leu Asn Gln Leu His Val Gln Leu Met Glu Leu Arg Ser Cys
805 810 815

Lys Gly Tyr Lys Gln Cys Asn Pro Arg Thr Arg Asn Met Asp Leu Gly
820 825 830

Leu Lys Asp Gly Gly Ser Tyr Glu Gln Tyr Arg Gln Phe Gln Arg Arg
835 840 845

Lys Trp Pro Glu Met Lys Arg Pro Ser Ser Lys Ser Leu Gly Gln Leu
850 855 860

Trp Glu Gly Trp Glu Gly
865 870

<210> 128
<211> 18
<212> PRT
<213> Homo sapiens

<400> 128
Met Val Arg Thr Leu Ser Leu Ala Val Leu Ser Trp Leu Pro Ala Ala
1 5 10 15

Val Cys

<210> 129
<211> 38
<212> PRT
<213> Homo sapiens

<400> 129
Glu Lys Lys Lys Lys Lys Lys Lys Arg Pro Gly Ala Val Ala His Ala
1 5 10 15

Leu Ile Pro Ala Leu Trp Glu Thr Glu Ala Gly Gly Ser Pro Glu Val
20 25 30

Gly Ser Ser Arg Pro Ala
35

<210> 130
<211> 60
<212> PRT
<213> Homo sapiens

<400> 130
Met Leu Ser Ala Val Leu Thr Met Leu Arg Phe Ile Ile Ala Phe Ser
1 5 10 15

Leu Leu Phe Cys Ser Cys Ser Thr Asp Lys His Cys Thr Trp Tyr His
20 25 30

Ala Leu Pro His Phe Lys Lys Ile Cys Leu Thr Glu Arg Lys Lys Met
35 40 45

Trp Phe Gly Leu Ala Ala Val Leu Ile Tyr Gly Ile
50 55 60

<210> 131
<211> 17

<212> PRT
<213> Homo sapiens

<400> 131
Ile Thr Phe Ser Cys Phe Phe Cys Asn Asn Cys Ser Gln Val Asn Leu
1   5   10   15

Gln

<210> 132
<211> 318
<212> PRT
<213> Homo sapiens

<400> 132
Met Arg Leu Leu Ala Gly Trp Leu Cys Leu Ser Leu Ala Ser Val Trp
1   5   10   15

Leu Ala Arg Arg Met Trp Thr Leu Arg Ser Pro Leu Thr Arg Ser Leu
            20   25   30

Tyr Val Asn Met Thr Ser Gly Pro Gly Gly Pro Ala Ala Ala Ala Gly
        35   40   45

Gly Arg Lys Glu Asn His Gln Trp Tyr Val Cys Asn Arg Glu Lys Leu
    50   55   60

Cys Glu Ser Leu Gln Ala Val Phe Val Gln Ser Tyr Leu Asp Gln Gly
65   70   75   80

Thr Gln Ile Phe Leu Asn Asn Ser Ile Glu Lys Ser Gly Trp Leu Phe
                85   90   95

Ile Gln Leu Tyr His Ser Phe Val Ser Ser Val Phe Ser Leu Phe Met
            100   105   110

Ser Arg Thr Ser Ile Asn Gly Leu Leu Gly Arg Gly Ser Met Phe Val
        115   120   125

Phe Ser Pro Asp Gln Phe Gln Arg Leu Leu Lys Ile Asn Pro Asp Trp
    130   135   140

Lys Thr His Arg Leu Leu Asp Leu Gly Ala Gly Asp Gly Glu Val Thr
145   150   155   160

Lys Ile Met Ser Pro His Phe Glu Glu Ile Tyr Ala Thr Glu Leu Ser
                165   170   175

Glu Thr Met Ile Trp Gln Leu Gln Lys Lys Lys Tyr Arg Val Leu Gly
            180   185   190

Ile Asn Glu Trp Gln Asn Thr Gly Phe Gln Tyr Asp Val Ile Ser Cys
        195   200   205

Leu Asn Leu Leu Asp Arg Cys Asp Gln Pro Leu Thr Leu Leu Lys Asp
    210   215   220

Ile Arg Ser Val Leu Glu Pro Thr Arg Gly Arg Val Ile Leu Ala Leu
225 230 235 240

Val Leu Pro Phe His Pro Tyr Val Glu Asn Val Gly Gly Lys Trp Glu
245 250 255

Lys Pro Ser Glu Ile Leu Glu Ile Lys Gly Gln Asn Trp Glu Glu Gln
260 265 270

Val Asn Ser Leu Pro Glu Val Phe Arg Lys Ala Gly Phe Val Ile Glu
275 280 285

Ala Phe Thr Arg Leu Pro Tyr Leu Cys Glu Gly Asp Met Tyr Asn Asp
290 295 300

Tyr Tyr Val Leu Asp Asp Ala Val Phe Val Leu Lys Pro Val
305 310 315

<210> 133
<211> 97
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (87)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (92)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 133
Leu Gly Ala Glu His Phe Lys Cys Ile Thr Trp Val Ala Gly Trp Ala
1 5 10 15

Val Pro Gly Leu Lys Gly Val Gly Ser Phe Phe Gln Gly Ala Pro Ser
20 25 30

Ala Ser Trp His Arg Thr Leu Ala Pro Ala His Pro Lys Leu Thr Leu
35 40 45

Val Gly Val Gly Pro Leu Thr Gln Thr Trp Pro Leu Pro Ser Leu Val
50 55 60

Leu Leu Pro Gln Leu Ser Pro Val Cys Gly Arg Val Cys Leu Asp Arg
65 70 75 80

Leu Trp Ala Gly Gln Gly Xaa Gly Gln Ala Glu Xaa Glu Phe Val Leu
85 90 95

Gly

<210> 134
<211> 35
<212> PRT
<213> Homo sapiens

<400> 134
Met Ser Leu Gly Phe Trp Val Trp Leu Pro Ser Cys Cys His Lys Met
1 5 10 15

Leu Val Val Thr Cys Thr Phe Gly His Tyr Leu Pro Leu Glu Ser Ser
20 25 30

His His Leu
35

<210> 135
<211> 93
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (13)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (39)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 135
Trp Phe Gln Thr Val Asp Arg His Cys Phe Val Leu Xaa Thr Asp Lys
1 5 10 15

Val Lys Leu Thr Trp Arg Asp Arg Phe Pro Ala Tyr Leu Thr Asn Leu
20 25 30

Val Ser Ile Ile Phe Met Xaa Ser Ser Arg Arg Leu Arg Pro Asp Glu
35 40 45

Val Arg Gly Asn Arg Lys Glu Val Ile Gly Phe Ser Arg Ala Trp Trp
50 55 60

Phe Thr Thr Val Ile Pro Ala Leu Trp Glu Ala Glu Ala Gly Arg Ser
65 70 75 80

Leu Glu Val Arg Ser Ser Arg Pro Ala Trp Pro Ile Trp
85 90

<210> 136
<211> 81
<212> PRT
<213> Homo sapiens

<220>
<221> SITE

<222> (67)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (76)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (81)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 136
Met Ile Leu Gly Ile His Trp Gly Ile Phe Leu Leu Leu Leu Leu Ser
1 5 10 15

Trp Leu Glu Leu Gln Arg Thr Val Ile Phe Phe Phe Ser Pro Phe Pro
20 25 30

Ile Gln Lys His Tyr Thr Leu Gly His Phe Ser Phe Ser Gln Arg Arg
35 40 45

Phe Met Asp Ser Gln Thr Glu Leu Cys Ala Thr Gly Lys Val Lys Arg
50 55 60

Glu Lys Xaa Ala Asp Glu Val Thr Trp Leu His Xaa Leu His His Ala
65 70 75 80

Xaa

<210> 137
<211> 9
<212> PRT
<213> Homo sapiens

<400> 137
Trp Gly Leu Leu Tyr Leu Glu Leu Asn
1 5

<210> 138
<211> 73
<212> PRT
<213> Homo sapiens

<400> 138
Ile Phe Leu Leu Leu Leu Leu Ser Trp Leu Glu Leu Gln Arg Thr Val
1 5 10 15

Ile Phe Phe Phe Ser Pro Phe Pro Ile Gln Lys His Tyr Thr Leu Gly
20 25 30

His Phe Ser Phe Ser Gln Arg Arg Phe Met Asp Ser Gln Thr Glu Leu
35 40 45

Cys Ala Thr Gly Lys Val Lys Arg Glu Lys Ala Ala Asp Glu Val Thr
50 55 60

Trp Leu His Val Leu His His Ala Glu
65 70

<210> 139
<211> 42
<212> PRT
<213> Homo sapiens

<400> 139
Met Phe Lys Lys Asp Leu Ile Cys Lys Arg Trp Ser Phe Phe Phe Trp
1 5 10 15

Gly Leu Leu Ile Ser Val Val Ile Leu Thr Ser Phe Ser Asn Tyr Ser
20 25 30

Arg Arg Phe Tyr Leu Asp Leu Tyr Phe Ser
35 40

<210> 140
<211> 7
<212> PRT
<213> Homo sapiens

<400> 140
Phe Ile Gly Phe Ile Leu Cys
1 5

<210> 141
<211> 49
<212> PRT
<213> Homo sapiens

<400> 141
Met Ser Pro Arg Pro Leu Ile Ala Arg Cys Glu Ala Leu Gly Cys Gly
1 5 10 15

Ala Arg Arg Leu Pro Trp Trp Ala Leu Ala Met Ala Leu Cys Ala Cys
20 25 30

Gly Arg Cys Val Ala Ala Asn Ser Ile Gly Glu Thr Leu Pro Ser Glu
35 40 45

Val

<210> 142
<211> 394
<212> PRT
<213> Homo sapiens

<400> 142

Val Thr Thr Leu Phe Leu Gly Pro Cys Tyr Cys Arg Gly Arg Leu His
1 5 10 15

Gly Leu Arg Gln Glu Ser Arg Leu Gly Asp Arg Ser Leu Val Ile Gly
20 25 30

Ala Gly Ala Cys Tyr Cys Ile Tyr Arg Leu Thr Arg Gly Arg Lys Gln
35 40 45

Asn Lys Glu Lys Met Ala Glu Gly Gly Ser Gly Asp Val Asp Asp Ala
50 55 60

Gly Asp Cys Ser Gly Ala Arg Tyr Asn Asp Trp Ser Asp Asp Asp Asp
65 70 75 80

Asp Ser Asn Glu Ser Lys Ser Ile Val Trp Tyr Pro Pro Trp Ala Arg
85 90 95

Ile Gly Thr Glu Ala Gly Thr Arg Ala Arg Ala Arg Ala Arg Ala Arg
100 105 110

Ala Thr Arg Ala Arg Arg Ala Val Gln Lys Arg Ala Ser Pro Asn Ser
115 120 125

Asp Asp Thr Val Leu Ser Pro Gln Glu Leu Gln Lys Val Leu Cys Leu
130 135 140

Val Glu Met Ser Glu Lys Pro Tyr Ile Leu Glu Ala Ala Leu Ile Ala
145 150 155 160

Leu Gly Asn Asn Ala Ala Tyr Ala Phe Asn Arg Asp Ile Ile Arg Asp
165 170 175

Leu Gly Gly Leu Pro Ile Val Ala Lys Ile Leu Asn Thr Arg Asp Pro
180 185 190

Ile Val Lys Glu Lys Ala Leu Ile Val Leu Asn Asn Leu Ser Val Asn
195 200 205

Ala Glu Asn Gln Arg Arg Leu Lys Val Tyr Met Asn Gln Val Cys Asp
210 215 220

Asp Thr Ile Thr Ser Arg Leu Asn Ser Ser Val Gln Leu Ala Gly Leu
225 230 235 240

Arg Leu Leu Thr Asn Met Thr Val Thr Asn Glu Tyr Gln His Met Leu
245 250 255

Ala Asn Ser Ile Ser Asp Phe Phe Arg Leu Phe Ser Ala Gly Asn Glu
260 265 270

Glu Thr Lys Leu Gln Val Leu Lys Leu Leu Leu Asn Leu Ala Glu Asn
275 280 285

Pro Ala Met Thr Arg Glu Leu Leu Arg Ala Gln Val Pro Ser Ser Leu
290 295 300

Gly Ser Leu Phe Asn Lys Lys Glu Asn Lys Glu Val Ile Leu Lys Leu

305 310 315 320

Leu Val Ile Phe Glu Asn Ile Asn Asp Asn Phe Lys Trp Glu Glu Asn
325 330 335

Glu Pro Thr Gln Asn Gln Phe Gly Glu Gly Ser Leu Phe Phe Phe Leu
340 345 350

Lys Glu Phe Gln Val Cys Ala Asp Lys Val Leu Gly Ile Glu Ser His
355 360 365

His Asp Phe Leu Val Lys Val Lys Val Gly Lys Phe Met Ala Lys Leu
370 375 380

Ala Glu His Met Phe Pro Lys Ser Gln Glu
385 390

<210> 143
<211> 85
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (8)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (33)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 143
Met Val Gly Leu Pro Ala Val Xaa Gln Leu Phe Trp Gly Leu Cys Leu
1 5 10 15

Cys Thr Cys Gly Leu Tyr Pro Ala Pro Gln Ser Trp Leu Ser Ser Gly
20 25 30

Xaa Tyr Lys Val Thr Ser Gly Ala Pro Ser Glu Arg Met Trp Pro Gln
35 40 45

Arg His Ala Ser Gly Phe Arg Leu Ser Gly Arg Thr Cys Leu Arg Ala
50 55 60

Thr Ala Pro Ser Pro Ser Phe Pro Phe Phe Ser Ala Val Ile Asn Leu
65 70 75 80

Ser Ala Cys Ser Lys
85

<210> 144
<211> 85
<212> PRT
<213> Homo sapiens

<400> 144
Pro Ser Val Ala Leu Cys Trp Ile Phe Phe Ile Pro Leu Gly Lys Trp
1 5 10 15

Glu Phe Phe Tyr Arg Pro Ala Ile Leu Leu Leu Cys Gln Ile Ala Leu
20 25 30

Tyr Tyr Gln Asp Thr Pro Met Ala His Phe Arg Leu Thr Glu Leu Phe
35 40 45

Leu Tyr Glu Cys Thr Val Val Ile Phe Trp Ala Val Cys Glu Phe Leu
50 55 60

Val Thr His Pro Leu Thr Thr Lys Ala Leu Ser Glu Gln Tyr Lys Ser
65 70 75 80

Ile Lys Ala Gln Ile
85

<210> 145
<211> 44
<212> PRT
<213> Homo sapiens

<400> 145
Met Ala Pro Ala Cys Gln Ile Leu Arg Trp Ala Leu Ala Leu Gly Leu
1 5 10 15

Gly Leu Met Phe Glu Val Thr His Ala Phe Arg Ser Gln Gly Arg Gly
20 25 30

Ser Leu Val Val Ala Val Gly Arg Glu Arg Lys Met
35 40

<210> 146
<211> 15
<212> PRT
<213> Homo sapiens

<400> 146
Pro Ala Pro Leu Pro Leu Arg Trp Ser Pro Ala Gly Pro Gly Gln
1 5 10 15

<210> 147
<211> 32
<212> PRT
<213> Homo sapiens

<400> 147
Trp Asn Pro Ile Ser Met Lys Asn Lys Leu Lys Ile Leu Lys Ile Lys
1 5 10 15

Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Gly Arg Pro
20 25 30

<210> 148
<211> 24
<212> PRT
<213> Homo sapiens

<400> 148
Met Trp His Leu Trp Arg Arg Leu Leu Ser Cys Phe Pro Val Ala Met
1 5 10 15

Leu Gln Asp Tyr Lys Tyr Ser Val
20

<210> 149
<211> 20
<212> PRT
<213> Homo sapiens

<400> 149
Ser Cys Leu Pro Val Gly Thr Asp Pro Gln Gln Met Gln Lys His Leu
1 5 10 15

Val Val Ile Lys
20

<210> 150
<211> 175
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (91)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (173)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (174)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (175)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 150
Val Phe Gly Met Leu Leu Gly Asp Thr Ile Ile Leu Asp Asn Leu Asp
1 5 10 15

Ala Ala Asn His Tyr Arg Lys Glu Val Val Lys Ile Thr His Cys Pro
20 25 30

Thr Leu Leu Thr Arg Asp Gly Asp Arg Ile Arg Ser Asn Gly Lys Phe
35 40 45

Gly Gly Leu Gln Asn Lys Ala Pro Pro Met Asp Lys Leu Arg Gly Met
50 55 60

Val Phe Gly Ala Pro Val Pro Lys Gln Cys Leu Ile Leu Gly Glu Gln
65 70 75 80

Ile Asp Leu Leu Gln Gln Tyr Arg Ser Ala Xaa Cys Lys Leu Asp Ser
85 90 95

Val Asn Lys Asp Leu Asn Ser Gln Leu Glu Tyr Leu Arg Thr Pro Asp
100 105 110

Met Arg Lys Lys Lys Gln Glu Leu Asp Glu His Glu Lys Asn Leu Lys
115 120 125

Leu Ile Glu Glu Lys Leu Gly Met Thr Pro Ile Arg Lys Cys Asn Asp
130 135 140

Ser Leu Arg His Ser Pro Lys Val Glu Thr Thr Asp Cys Pro Val Pro
145 150 155 160

Pro Lys Arg Met Arg Arg Glu Ala Gly Asp Lys Arg Xaa Xaa Xaa
165 170 175

<210> 151
<211> 156
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (102)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 151
Val Val Lys Ile Thr His Cys Pro Thr Leu Leu Thr Arg Asp Gly Asp
1 5 10 15

Arg Ile Arg Ser Asn Gly Lys Phe Gly Gly Leu Gln Asn Lys Ala Pro
20 25 30

Pro Met Asp Lys Leu Arg Gly Met Val Phe Gly Ala Pro Val Pro Lys
35 40 45

Gln Cys Leu Ile Leu Gly Glu Gln Ile Asp Leu Leu Gln Gln Tyr Arg
50 55 60

Ser Ala Val Cys Lys Leu Asp Ser Val Asn Lys Asp Leu Asn Ser Gln
65 70 75 80

Leu Glu Tyr Leu Arg Thr Pro Asp Met Arg Lys Lys Lys Gln Glu Leu
85 90 95

Asp Glu His Glu Lys Xaa Leu Lys Leu Ile Glu Glu Lys Leu Gly Met
100 105 110

Thr Pro Ile Arg Lys Cys Asn Asp Ser Leu Arg His Ser Pro Lys Val
115 120 125

Glu Thr Thr Asp Cys Pro Val Pro Pro Lys Arg Met Arg Arg Glu Ala
130 135 140

Thr Arg Gln Asn Arg Ile Ile Thr Lys Thr Asp Val
145 150 155

<210> 152
<211> 37
<212> PRT
<213> Homo sapiens

<400> 152
Met Val Glu Ser Pro Val Cys Gly Leu Leu Glu Gly Trp Phe Phe Leu
1 5 10 15

Leu Phe Ser Leu Ala Phe Leu Ser Thr His Leu Phe Ser Glu Ala Ser
20 25 30

Pro Leu Ser Ile Leu
35

<210> 153
<211> 58
<212> PRT
<213> Homo sapiens

<400> 153
Met Thr Leu Ser Val Leu Gln His Phe Phe Ile Cys Val Leu Leu Ile
1 5 10 15

Leu Leu Leu Asp Thr Asn Leu Cys Arg Gln Ile Ser Ser His Ser Phe
20 25 30

Glu Phe Ser Gly Asn Gln Pro Leu Val Phe Cys Cys Ile Ser Ser Ile
35 40 45

Ser Ala Lys Leu Val Leu Asp Gln Ala Gly
50 55

<210> 154
<211> 2
<212> PRT
<213> Homo sapiens

<400> 154
Leu Glu

1

<210> 155
<211> 283
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (174)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (189)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (205)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 155
Met Lys Ile Val Pro Leu Thr Ala Ala Val Leu Ala Leu Val Leu Ala
1 5 10 15

Pro Ala Ala His Ala Gln Pro Ala Asn Lys Ala Thr Thr Val Ser Pro
20 25 30

Thr Ala Ala Ala Phe Leu Ala Gln Phe Ala Thr Glu Gly Asn Asp Ser
35 40 45

Val Ser Trp Ala Gln Phe Glu Ala Phe Arg Lys Gln Arg Tyr Ala Asp
50 55 60

Thr Asp Arg Asn Gln Asp Gly His Val Asp Glu Gln Glu Tyr Val Asp
65 70 75 80

Glu Tyr Leu Gln Arg Phe Asp Val Arg Leu Ala Asp Ala Arg Ala Gly
85 90 95

His Leu Arg Gln Thr Asp Thr Arg Phe Lys Ala Leu Asp Arg Asp Gly
100 105 110

Asn Gly Ala Ile Ser Arg Ala Glu Tyr Asp Ala Ala Gly Glu Arg Thr
115 120 125

Trp Ala Gly Tyr Glu Arg Ser Gln Asn Ala Thr Gln Glu Thr Ala Ala
130 135 140

Ala Ser Ser Arg Asp Pro Leu Lys Met Pro Thr Ser His Thr Ala Asn
145 150 155 160

Gly Met Leu Asp Leu Tyr Asp Arg Asn Lys Asp Gly Ala Xaa Asp Arg
165 170 175

Glu Glu Phe Asp Ala Val Arg Ala Ala Ser Phe Ala Xaa Thr Asp Thr

180 185 190

Asp Gly Asn Gly Thr Leu Ser Leu Ala Glu Tyr Thr Xaa Glu Phe Glu
195 200 205

Gly Arg Leu Asp Gln Gln Arg Gln Arg Val Arg Ala Asp Ala Glu Arg
210 215 220

Gln Ala Arg Val Arg Phe Ala Ser Leu Asp Lys Asp Thr Asp Gly Arg
225 230 235 240

Met Thr Phe Ala Glu Tyr Gln Leu Ser Gly Lys Arg Met Phe Asp Arg
245 250 255

Ala Asp Ser Asn Gly Asp Gly Val Val Asp Ala Arg Asp Pro Glu Pro
260 265 270

Val Ala Gly Ala His Ser Ala Asn Gly Asn Arg
275 280

<210> 156
<211> 124
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (89)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (103)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (104)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (113)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (121)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 156
Val Ala Gln Val Gln Val Pro Gly Gly His Ile Gly Leu Gly Tyr Leu
1 5 10 15

Ala Arg Ile Asp Phe His Arg Arg Asp Gly Thr Gly Gly Ile Pro Ala
20 25 30

```
Arg Ile Asp Gly Gly Glu Ile Asp Val Ala Leu Leu Pro Gly Gln Ala
         35                  40                  45

Val Asp His Ile Met Ala Arg Ala Cys Gly Gly Glu His Leu Ala Glu
     50                  55                  60

Val Gly Arg Gly Thr Val Gln Gly Leu Leu Gly Arg Ala Val Leu Ala
 65                  70                  75                  80

Ala Gln Ala Arg Arg Ala Pro Pro Xaa Gln Pro Leu Pro Ala Thr Met
                 85                  90                  95

Gly Phe Trp Gly Trp Lys Xaa Xaa Pro Asn Arg Gly Leu Trp Phe Lys
            100                 105                 110

Xaa Trp Lys Pro Pro Phe Gly Ala Xaa Gly Val Pro
        115                 120
```

<210> 157
<211> 42
<212> PRT
<213> Homo sapiens

<400> 157

```
Met Leu Leu Ser Trp Thr Val Leu Ile Ile Ile Leu Pro Phe Ala Gly
  1               5                  10                  15

Asp Val Ser Ser His Leu Cys Ile Leu Arg Pro Phe Ala Gly Ser Val
             20                  25                  30

Ser Ser Cys Leu Ser Asn Phe Lys Arg Ile
         35                  40
```

<210> 158
<211> 29
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (3)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (13)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (17)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (19)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (23)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 158
Met Leu Xaa Ser Asn Ser Phe Ser Pro Ser Leu Ser Xaa Tyr Leu Cys
1 5 10 15

Xaa Leu Xaa Phe Ser Leu Xaa Ser Ser Lys Ser Ser Lys
20 25

<210> 159
<211> 332
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (204)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (283)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (305)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 159
Met Glu Val Arg Lys Leu Ser Ile Ser Trp Gln Phe Leu Ile Val Leu
1 5 10 15

Val Leu Ile Leu Gln Ile Leu Ser Ala Leu Asp Phe Asp Pro Tyr Arg
20 25 30

Val Leu Gly Val Ser Arg Thr Ala Ser Gln Ala Asp Ile Lys Lys Ala
35 40 45

Tyr Lys Lys Leu Ala Arg Glu Trp His Pro Asp Lys Asn Lys Asp Pro
50 55 60

Gly Ala Glu Asp Lys Phe Ile Gln Ile Ser Lys Ala Tyr Glu Ile Leu
65 70 75 80

Ser Asn Glu Glu Lys Arg Ser Asn Tyr Asp Gln Tyr Gly Asp Ala Gly
85 90 95

Glu Asn Gln Gly Tyr Gln Lys Gln Gln Gln Gln Arg Glu Tyr Arg Phe
100 105 110

Arg His Phe His Glu Asn Phe Tyr Phe Asp Glu Ser Phe Phe His Phe

115 120 125

Pro Phe Asn Ser Glu Arg Arg Asp Ser Ile Asp Glu Lys Tyr Leu Leu
130 135 140

His Phe Ser His Tyr Val Asn Glu Val Val Pro Asp Ser Phe Lys Lys
145 150 155 160

Pro Tyr Leu Ile Lys Ile Thr Ser Asp Trp Cys Phe Ser Cys Ile His
165 170 175

Ile Glu Pro Val Trp Lys Glu Val Ile Gln Glu Leu Glu Glu Leu Gly
180 185 190

Val Gly Ile Gly Val Val His Ala Gly Tyr Glu Xaa Arg Leu Ala His
195 200 205

His Leu Gly Ala His Ser Thr Pro Ser Ile Leu Gly Ile Ile Asn Gly
210 215 220

Lys Ile Ser Phe Phe His Asn Ala Val Val Arg Glu Asn Leu Arg Gln
225 230 235 240

Phe Val Glu Ser Leu Leu Pro Gly Asn Leu Val Glu Lys Val Thr Asn
245 250 255

Lys Asn Tyr Val Arg Phe Leu Ser Gly Trp Gln Gln Glu Asn Lys Pro
260 265 270

His Val Leu Leu Phe Asp Gln Thr Pro Ile Xaa Pro Leu Leu Tyr Lys
275 280 285

Leu Thr Ala Phe Ala Tyr Lys Asp Tyr Leu Ser Phe Gly Tyr Val Tyr
290 295 300

Xaa Gly Leu Arg Gly Thr Glu Glu Met Thr Arg Arg Tyr Asn Ile Asn
305 310 315 320

Ile Tyr Ala Pro Thr Leu Leu Ala Leu Lys Asn Ile
325 330

<210> 160
<211> 204
<212> PRT
<213> Homo sapiens

<400> 160
Met Met Pro Leu Leu Ser Leu Ile Phe Ser Ala Leu Phe Ile Leu Phe
1 5 10 15

Gly Thr Val Ile Val Gln Ala Phe Ser Asp Ser Asn Asp Glu Arg Glu
20 25 30

Ser Ser Pro Pro Glu Lys Glu Glu Ala Gln Glu Lys Thr Gly Lys Thr
35 40 45

Glu Pro Ser Phe Thr Lys Glu Asn Ser Ser Lys Ile Pro Lys Lys Gly

50 55 60

Phe Val Glu Val Thr Glu Leu Thr Asp Val Thr Tyr Thr Ser Asn Leu
65 70 75 80

Val Arg Leu Arg Pro Gly His Met Asn Val Val Leu Ile Leu Ser Asn
85 90 95

Ser Thr Lys Thr Ser Leu Leu Gln Lys Phe Ala Leu Glu Val Tyr Thr
100 105 110

Phe Thr Gly Ser Ser Cys Leu His Phe Ser Phe Leu Ser Leu Asp Lys
115 120 125

His Arg Glu Trp Leu Glu Tyr Leu Leu Glu Phe Ala Gln Asp Ala Ala
130 135 140

Pro Ile Pro Asn Gln Tyr Asp Lys His Phe Met Glu Arg Asp Tyr Thr
145 150 155 160

Gly Tyr Val Leu Ala Leu Asn Gly His Lys Lys Tyr Phe Cys Leu Phe
165 170 175

Lys Pro Gln Lys Thr Val Glu Glu Glu Glu Ala Ile Gly Ser Cys Ser
180 185 190

Asp Val Asp Ser Ser Leu Tyr Leu Gly Glu Ser Arg
195 200

<210> 161
<211> 42
<212> PRT
<213> Homo sapiens

<400> 161
Met Asn Ser Ser Phe Phe Ile Ser Leu Pro Ala Leu Ile Trp Ser Val
1 5 10 15

Cys Leu Ile Leu Gly Trp Trp Gln Val Ser Ser Gly Lys Val Ala His
20 25 30

Cys Gly Phe Ile Phe Cys Phe Pro Asn Asn
35 40

<210> 162
<211> 111
<212> PRT
<213> Homo sapiens

<400> 162
Cys Gly Ser His Arg Met Ser Trp Lys Met Tyr Cys Pro Leu His Phe
1 5 10 15

Ser Gly Arg Val Cys Glu Glu Leu Lys Phe Phe Phe Ser Phe Phe Phe
20 25 30

```
Phe Leu Arg Arg Ser Leu Thr Pro Ala Gln Ala Thr Ala Gly Asp Ser
        35                  40                  45

Val Ser Lys Lys Gln Arg Glu Glu Arg Lys Lys Glu Lys Lys Glu Gly
    50                  55                  60

Arg Arg Lys Glu Gly Arg Asn Glu Gly Thr Lys Glu Gly Arg Lys Arg
65                  70                  75                  80

Lys Glu Gly Arg Lys Lys Glu Arg Glu Arg Glu Arg Lys Lys Glu Arg
                85                  90                  95

Lys Lys Glu Arg Lys Lys Glu Lys Lys Lys Lys Lys Thr Gly Thr
            100                 105                 110


<210> 163
<211> 128
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (67)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (68)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (70)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (96)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 163
Met Pro Val Phe Val Cys Ser Ile Gly Leu Cys Phe Leu Phe Ser Ile
 1               5                  10                  15

Leu Leu Leu Phe Pro Pro Phe Gln Phe Ser Tyr Ile Cys Trp Leu Ser
            20                  25                  30

Gln Ala Ser Val Tyr Ser Pro Ser Pro Ser Leu Ser Asn Leu Glu Val
        35                  40                  45

Leu Leu Cys Leu Ser Ile Leu Leu Met Ile Ile Phe Pro Phe Leu Ile
    50                  55                  60

Ser Ile Xaa Xaa Ile Xaa Ser Ile Gly Arg Leu Ser Thr His Met Gly
65                  70                  75                  80

Ala His Thr His Thr His Thr His Thr His Thr His Thr His Thr Xaa
```

85 90 95

Val Cys Tyr Trp Pro Leu Leu Leu Ile Ser Gln Glu Asn Glu Pro Phe
100 105 110

Arg Met Phe Leu Pro Leu His Ser Ala Leu Thr Gln Asn Phe Cys Ser
115 120 125

<210> 164
<211> 57
<212> PRT
<213> Homo sapiens

<400> 164
Met Pro Pro His Arg Gln Thr Asp Gly Gln Met Gly Leu Pro Ala Pro
1 5 10 15

Ala Leu Trp Val Trp Gly Leu Leu Leu Ser Ser Ser Phe Gln Thr Leu
20 25 30

Leu Pro Ala Phe Pro Lys Pro Pro Ala Leu Asn Leu Gly Cys Ser Thr
35 40 45

Arg Pro Ile Pro Ser Phe Leu Lys Ile
50 55

<210> 165
<211> 93
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (24)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (65)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 165
Gln Val Ser Leu Pro Thr Arg Leu Leu Gln Met Pro Gly Met Gly Leu
1 5 10 15

Asp Ser Arg Phe Gln Ala Trp Xaa Pro Ser Pro Tyr Leu Gly Pro Gln
20 25 30

Pro Arg Ala Pro Arg Pro Gly Leu Gln Pro Gly Pro Ser Leu Arg Gly
35 40 45

Ala Glu Phe Arg Glu Ser Cys Pro Arg Ser Gln Lys Arg Gly Arg Glu
50 55 60

Xaa Gly Arg Pro Cys Pro Gly Cys Arg Pro Gly Gly Trp Gly Leu Pro
65 70 75 80

Ala Arg Leu Gly Gln Pro Gln Leu Gln Thr Gly Pro Gly
85 90

<210> 166
<211> 24
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (9)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (18)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 166
Met Ser Trp Arg Val Trp Ala Leu Xaa Phe Phe Pro Ala Val Cys Val
1 5 10 15

Cys Xaa Cys Val Cys Val Tyr Thr
20

<210> 167
<211> 65
<212> PRT
<213> Homo sapiens

<400> 167
Val Leu Met Arg Ser Asp Gly Phe Ile Arg Gly Phe Ser Pro Phe Cys
1 5 10 15

Trp Ala Leu Leu Leu Leu Pro Pro Arg Glu Glu Gly Cys Val Cys Phe
20 25 30

Pro Phe Cys His Asp Cys Lys Phe Pro Val Ala Ser Pro Ser Leu Arg
35 40 45

Asn Cys Glu Ser Ile Lys Ala Leu Phe Phe Ile Lys Lys Lys Lys Lys
50 55 60

Asn
65

<210> 168
<211> 12
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (8)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 168
Leu Phe Ser Gly Trp Leu Val Xaa Leu Cys Gly Val
1 5 10

<210> 169
<211> 86
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (4)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (11)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 169
Asn Leu Trp Xaa Ala His Phe Phe Leu Asn Xaa Ser Ser Ile Gln Ile
1 5 10 15

Glu Tyr Pro Pro Leu Ser Lys Met Leu Glu Thr Pro Lys Gly Lys Gly
20 25 30

Trp Phe Phe Gly Glu Phe Phe Phe Trp Val Phe Leu Phe Phe Leu Gly
35 40 45

Phe Ala Phe Gly Phe Trp Asn Ser Leu Phe Val Leu Tyr Leu Phe Val
50 55 60

Gly His Pro Lys Ser Glu Ile Cys Ser Lys Ile Gln Asn Val Lys Cys
65 70 75 80

Ser Ser Glu His Phe Leu
85

<210> 170
<211> 25
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (13)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 170
Met Val Gly Arg Cys Ser Ile Leu Ser Ser Thr Pro Xaa Arg His Pro
1 5 10 15

Ser Leu Ser Trp Glu Gly Leu Gly Gly
20 25

<210> 171
<211> 314
<212> PRT
<213> Homo sapiens

<400> 171
Cys Gly Lys Gly His Arg Cys Val Asn Ser Pro Gly Ser Phe Arg Cys
1 5 10 15

Glu Cys Lys Thr Gly Tyr Tyr Phe Asp Gly Ile Ser Arg Met Cys Val
20 25 30

Asp Val Asn Glu Cys Gln Arg Tyr Pro Gly Arg Leu Cys Gly His Lys
35 40 45

Cys Glu Asn Thr Leu Gly Ser Tyr Leu Cys Ser Cys Ser Val Gly Phe
50 55 60

Arg Leu Ser Val Asp Gly Arg Ser Cys Glu Asp Ile Asn Glu Cys Ser
65 70 75 80

Ser Ser Pro Cys Ser Gln Glu Cys Ala Asn Val Tyr Gly Ser Tyr Gln
85 90 95

Cys Tyr Cys Arg Arg Gly Tyr Gln Leu Ser Asp Val Asp Gly Val Thr
100 105 110

Cys Glu Asp Ile Asp Glu Cys Ala Leu Pro Thr Gly Gly His Ile Cys
115 120 125

Ser Tyr Arg Cys Ile Asn Ile Pro Gly Ser Phe Gln Cys Ser Cys Pro
130 135 140

Ser Ser Gly Tyr Arg Leu Ala Pro Asn Gly Arg Asn Cys Gln Asp Ile
145 150 155 160

Asp Glu Cys Val Thr Gly Ile His Asn Cys Ser Ile Asn Glu Thr Cys
165 170 175

Phe Asn Ile Gln Gly Ala Phe Arg Cys Leu Ala Phe Glu Cys Pro Glu
180 185 190

Asn Tyr Arg Arg Ser Ala Ala Thr Arg Cys Glu Arg Leu Pro Cys His
195 200 205

Glu Asn Arg Glu Cys Ser Lys Leu Pro Leu Arg Ile Thr Tyr Tyr His
210 215 220

Leu Ser Phe Pro Thr Asn Ile Gln Ala Pro Ala Val Val Phe Arg Met
225 230 235 240

Gly Pro Ser Ser Ala Val Pro Gly Asp Ser Met Gln Leu Ala Ile Thr
245 250 255

Gly Gly Asn Glu Glu Gly Phe Phe Thr Thr Arg Lys Val Ser Pro His
260 265 270

Ser Gly Val Val Ala Leu Thr Lys Pro Val Pro Glu Pro Arg Asp Leu
275 280 285

Leu Leu Thr Val Lys Met Asp Leu Ser Arg His Gly Thr Val Ser Ser
290 295 300

Phe Val Ala Lys Leu Phe Ile Phe Val Ser
305 310

<210> 172
<211> 329
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (252)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 172
Cys Asn Pro Gly Phe Arg Leu Val Gly Pro Ser Ser Val Val Cys Leu
1 5 10 15

Pro Asn Gly Thr Trp Thr Gly Glu Gln Pro His Cys Arg Gly Ile Ser
20 25 30

Glu Cys Ser Ser Gln Pro Cys Gln Asn Gly Gly Thr Cys Val Glu Gly
35 40 45

Val Asn Gln Tyr Arg Cys Ile Cys Pro Pro Gly Arg Thr Gly Asn Arg
50 55 60

Cys Gln His Gln Ala Gln Thr Ala Ala Pro Glu Gly Ser Val Ala Gly
65 70 75 80

Asp Ser Ala Phe Ser Arg Ala Pro Arg Cys Ala Gln Val Glu Arg Ala
85 90 95

Gln His Cys Ser Cys Glu Ala Gly Phe His Leu Ser Gly Ala Ala Gly
100 105 110

Asp Ser Val Cys Gln Asp Val Asn Glu Cys Glu Leu Tyr Gly Gln Glu
115 120 125

Gly Arg Pro Arg Leu Cys Met His Ala Cys Val Asn Thr Pro Gly Ser
130 135 140

Tyr Arg Cys Thr Cys Pro Gly Gly Tyr Arg Thr Leu Ala Asp Gly Lys
145 150 155 160

Ser Cys Glu Asp Val Asp Glu Cys Val Gly Leu Gln Pro Val Cys Pro
165 170 175

Gln Gly Thr Thr Cys Ile Asn Thr Gly Gly Ser Phe Gln Cys Val Ser
180 185 190

Pro Glu Cys Pro Glu Gly Ser Gly Asn Val Ser Tyr Val Lys Thr Ser
195 200 205

Pro Phe Gln Cys Glu Arg Asn Pro Cys Pro Met Asp Ser Arg Pro Cys
210 215 220

Arg His Leu Pro Lys Thr Ile Ser Phe His Tyr Leu Ser Leu Pro Ser
225 230 235 240

Asn Leu Lys Thr Pro Ile Thr Leu Phe Arg Met Xaa Thr Ala Ser Ala
245 250 255

Pro Gly Arg Ala Gly Pro Asn Ser Leu Arg Phe Gly Ile Val Gly Gly
260 265 270

Asn Ser Arg Gly His Phe Val Met Gln Arg Ser Asp Arg Gln Thr Gly
275 280 285

Asp Leu Ile Leu Val Gln Asn Leu Glu Gly Pro Gln Thr Leu Glu Val
290 295 300

Asp Val Asp Met Ser Glu Tyr Leu Asp Arg Ser Phe Gln Ala Asn His
305 310 315 320

Val Ser Lys Val Thr Ile Phe Val Ser
325

<210> 173
<211> 209
<212> PRT
<213> Homo sapiens

<400> 173
His Glu Leu Gln Asp Thr Val Ala Leu Asp His Gly Gly Cys Cys Pro
1 5 10 15

Ala Leu Ser Arg Leu Val Pro Arg Gly Phe Gly Thr Glu Met Trp Thr
20 25 30

Leu Phe Ala Leu Ser Gly Pro Leu Phe Leu Phe Gln Val Leu Thr Phe
35 40 45

Met Ile Tyr Ile Val Ser Thr Val Phe Cys Gly His Leu Gly Lys Val
50 55 60

Glu Leu Ala Ser Val Thr Leu Ala Val Ala Phe Val Asn Val Cys Gly
65 70 75 80

Val Ser Val Gly Val Gly Leu Ser Ser Ala Cys Asp Thr Leu Met Ser
85 90 95

Gln Ser Phe Gly Ser Pro Asn Lys Lys His Val Gly Val Ile Leu Gln
100 105 110

Arg Gly Ala Leu Val Leu Leu Leu Cys Cys Leu Pro Cys Trp Ala Leu
115 120 125

Phe Leu Asn Thr Gln His Ile Leu Leu Leu Phe Arg Gln Asp Pro Asp
130 135 140

Val Ser Arg Leu Thr Gln Asp Tyr Val Met Ile Phe Ile Pro Gly Leu
145 150 155 160

Pro Val Ile Phe Leu Tyr Asn Leu Leu Ala Lys Tyr Leu Gln Asn Gln
165 170 175

Val Gln Val Phe Glu Cys Val Gly Arg Pro Phe Ser Gln His Thr Ala
180 185 190

Leu Phe Gln Trp Glu Gly Gly Leu Gly Leu Ser Pro Ser Leu His His
195 200 205

Leu

<210> 174
<211> 151
<212> PRT
<213> Homo sapiens

<400> 174
Phe Gly Thr Glu Met Trp Thr Leu Phe Ala Leu Ser Gly Pro Leu Phe
1 5 10 15

Leu Phe Gln Val Leu Thr Phe Met Ile Tyr Ile Val Ser Thr Val Phe
20 25 30

Cys Gly His Leu Gly Lys Val Glu Leu Ala Ser Val Thr Leu Ala Val
35 40 45

Ala Phe Val Asn Val Cys Gly Val Ser Val Gly Val Gly Leu Ser Ser
50 55 60

Ala Cys Asp Thr Leu Met Ser Gln Ser Phe Gly Ser Pro Asn Lys Lys
65 70 75 80

His Val Gly Val Ile Leu Gln Arg Gly Ala Leu Val Leu Leu Leu Cys
85 90 95

Cys Leu Pro Cys Trp Ala Leu Phe Leu Asn Thr Gln His Ile Leu Leu
100 105 110

Leu Phe Arg Gln Asp Pro Asp Val Ser Arg Leu Thr Gln Asp Tyr Val
115 120 125

Met Ile Phe Ile Pro Gly Leu Pro Val Ile Phe Leu Tyr Asn Leu Leu
130 135 140

Ala Lys Tyr Leu Gln Asn Gln
145 150

<210> 175
<211> 122
<212> PRT
<213> Homo sapiens

<400> 175
Gln Val Leu Ser Gly Val Val Gly Asn Cys Val Asn Gly Val Ala Asn
1 5 10 15

Tyr Ala Leu Val Ser Val Leu Asn Leu Gly Val Arg Gly Ser Ala Tyr
20 25 30

Ala Asn Ile Ile Ser Gln Phe Ala Gln Thr Val Phe Leu Leu Leu Tyr
35 40 45

Ile Val Leu Lys Lys Leu His Leu Glu Thr Trp Ala Gly Trp Ser Ser
50 55 60

Gln Cys Leu Gln Asp Trp Gly Pro Phe Phe Ser Leu Ala Val Pro Ser
65 70 75 80

Met Leu Met Ile Cys Val Glu Trp Trp Ala Tyr Glu Ile Gly Ser Phe
85 90 95

Leu Met Gly Leu Leu Ser Val Val Asp Leu Ser Ala Gln Ala Val Ile
100 105 110

Tyr Glu Val Ala Thr Val Thr Tyr Met Val
115 120

<210> 176
<211> 33
<212> PRT
<213> Homo sapiens

<400> 176
Gly Asp Thr Gly Glu Ile Lys Ser Glu Val Arg Glu Gln Ile Asn Ala
1 5 10 15

Lys Val Ala Glu Trp Arg Glu Glu Gly Lys Ala Glu Ile Ile Pro Gly
20 25 30

Val

<210> 177
<211> 40
<212> PRT
<213> Homo sapiens

<400> 177
Val Leu Phe Ile Asp Glu Val His Met Leu Asp Ile Glu Ser Phe Ser
1 5 10 15

Phe Leu Asn Arg Ala Leu Glu Ser Asp Met Ala Pro Val Leu Ile Met

20 25 30

Ala Thr Asn Arg Gly Ile Thr Arg
35 40

<210> 178
<211> 431
<212> PRT
<213> Homo sapiens

<400> 178
Asn Val Ile Leu Ile Leu Thr Asp Asp Gln Asp Ile Glu Leu Gly Ser
1 5 10 15

Met Asp Phe Met Pro Lys Thr Ser Gln Ile Met Lys Glu Arg Gly Thr
20 25 30

Glu Phe Thr Ser Gly Tyr Val Thr Thr Pro Ile Cys Cys Pro Ser Arg
35 40 45

Ser Thr Ile Leu Thr Gly Leu Tyr Val His Asn His His Val His Thr
50 55 60

Asn Asn Gln Asn Cys Thr Gly Val Glu Trp Arg Lys Val His Glu Lys
65 70 75 80

Lys Ser Ile Gly Val Tyr Leu Gln Glu Ala Gly Tyr Arg Thr Ala Tyr
85 90 95

Leu Gly Lys Tyr Leu Asn Glu Tyr Asp Gly Ser Tyr Ile Pro Pro Gly
100 105 110

Trp Asp Glu Trp His Ala Ile Val Lys Asn Ser Lys Phe Tyr Asn Tyr
115 120 125

Thr Met Asn Ser Asn Gly Glu Arg Glu Lys Phe Gly Ser Glu Tyr Glu
130 135 140

Lys Asp Tyr Phe Thr Asp Leu Val Thr Asn Arg Ser Leu Lys Phe Ile
145 150 155 160

Asp Lys His Ile Lys Ile Arg Ala Trp Gln Pro Phe Ala Leu Ile Ile
165 170 175

Ser Tyr Pro Ala Pro His Gly Pro Glu Asp Pro Ala Pro Gln Phe Ala
180 185 190

His Met Phe Glu Asn Glu Ile Ser His Arg Thr Gly Ser Trp Asn Phe
195 200 205

Ala Pro Asn Pro Asp Lys Gln Trp Leu Leu Gln Arg Thr Gly Lys Met
210 215 220

Asn Asp Val His Ile Ser Phe Thr Asp Leu Leu His Arg Arg Arg Leu
225 230 235 240

Gln Thr Leu Gln Ser Val Asp Glu Gly Ile Glu Arg Leu Phe Asn Leu

245 250 255

Leu Arg Glu Leu Asn Gln Leu Trp Asn Thr Tyr Ala Ile Tyr Thr Ser
260 265 270

Asp His Gly Tyr His Leu Gly Gln Phe Gly Leu Leu Lys Gly Lys Asn
275 280 285

Met Pro Tyr Glu Phe Asp Ile Arg Val Pro Phe Phe Met Arg Gly Pro
290 295 300

Gly Ile Pro Arg Asn Val Thr Phe Asn Glu Ile Val Thr Asn Val Asp
305 310 315 320

Ile Ala Pro Thr Met Leu His Ile Ala Gly Val Pro Lys Pro Ala Arg
325 330 335

Met Asn Gly Arg Ser Leu Leu Glu Leu Val Ala Leu Lys Lys Lys Lys
340 345 350

Lys Lys His Met Thr Ala Leu Lys Pro Trp Arg Asp Thr Ile Leu Ile
355 360 365

Glu Arg Gly Lys Met Pro Lys Leu Lys Lys Ile Arg Asp Arg Tyr Ile
370 375 380

Lys Gln Lys Lys Lys Phe Asn Lys Glu Asn Arg Leu Ser Lys Glu Cys
385 390 395 400

Lys Arg Arg Lys Trp Gln Arg Asp Cys Val His Gly Gln Leu Trp Lys
405 410 415

Cys Tyr Tyr Thr Val Glu Asp Arg Trp Arg Ile Tyr Lys Cys Arg
420 425 430

<210> 179
<211> 434
<212> PRT
<213> Homo sapiens

<400> 179
Asn Ile Ile Leu Val Leu Thr Asp Asp Gln Asp Val Glu Leu Gly Ser
1 5 10 15

Met Gln Val Met Asn Lys Thr Arg Arg Ile Met Glu Gln Gly Gly Ala
20 25 30

His Phe Ile Asn Ala Phe Val Thr Thr Pro Met Cys Cys Pro Ser Arg
35 40 45

Ser Ser Ile Leu Thr Gly Lys Tyr Val His Asn His Asn Thr Tyr Thr
50 55 60

Asn Asn Glu Asn Cys Ser Ser Pro Ser Trp Gln Ala Gln His Glu Ser
65 70 75 80

Arg Thr Phe Ala Val Tyr Leu Asn Ser Thr Gly Tyr Arg Thr Ala Phe

```
                85                  90                  95

Phe Gly Lys Tyr Leu Asn Glu Tyr Asn Gly Ser Tyr Val Pro Pro Gly
            100                 105                 110

Trp Lys Glu Trp Val Gly Leu Leu Lys Asn Ser Arg Phe Tyr Asn Tyr
        115                 120                 125

Thr Leu Cys Arg Asn Gly Val Lys Glu Lys His Gly Ser Asp Tyr Ser
    130                 135                 140

Lys Asp Tyr Leu Thr Asp Leu Ile Thr Asn Asp Ser Val Ser Phe Phe
145                 150                 155                 160

Arg Thr Ser Lys Lys Met Tyr Pro His Arg Pro Val Leu Met Val Ile
                165                 170                 175

Ser His Ala Ala Pro His Gly Pro Glu Asp Ser Ala Pro Gln Tyr Ser
            180                 185                 190

Arg Leu Phe Pro Asn Ala Ser Gln His Ile Thr Pro Ser Tyr Asn Tyr
        195                 200                 205

Ala Pro Asn Pro Asp Lys His Trp Ile Met Arg Tyr Thr Gly Pro Met
    210                 215                 220

Lys Pro Ile His Met Glu Phe Thr Asn Met Leu Gln Arg Lys Arg Leu
225                 230                 235                 240

Gln Thr Leu Met Ser Val Asp Asp Ser Met Glu Thr Ile Tyr Asn Met
                245                 250                 255

Leu Val Glu Thr Gly Glu Leu Asp Asn Thr Tyr Ile Val Tyr Thr Ala
            260                 265                 270

Asp His Gly Tyr His Ile Gly Gln Phe Gly Leu Val Lys Gly Lys Ser
        275                 280                 285

Met Pro Tyr Glu Phe Asp Ile Arg Val Pro Phe Tyr Val Arg Gly Pro
    290                 295                 300

Asn Val Glu Ala Gly Cys Leu Asn Pro His Ile Val Leu Asn Ile Asp
305                 310                 315                 320

Leu Ala Pro Thr Ile Leu Asp Ile Ala Gly Leu Asp Ile Pro Ala Asp
                325                 330                 335

Met Asp Gly Lys Ser Ile Leu Lys Leu Leu Asp Thr Glu Arg Pro Val
            340                 345                 350

Asn Arg Phe His Leu Lys Lys Lys Met Arg Val Trp Arg Asp Ser Phe
        355                 360                 365

Leu Val Glu Arg Gly Lys Leu Leu His Lys Arg Asp Asn Asp Lys Val
    370                 375                 380

Asp Ala Gln Glu Glu Asn Phe Leu Pro Lys Tyr Gln Arg Val Lys Asp
385                 390                 395                 400
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Leu Cys Gln Arg Ala Glu Tyr Gln Thr Ala Cys Glu Gln Leu Gly Gln
405 410 415

Lys Trp Gln Cys Val Glu Asp Ala Thr Gly Lys Leu Lys Leu His Lys
420 425 430

Cys Lys

<210> 180
<211> 283
<212> PRT
<213> Homo sapiens

<400> 180
Met Thr Ser Gly Pro Gly Gly Pro Ala Ala Ala Ala Gly Gly Arg Lys
1 5 10 15

Glu Asn His Gln Trp Tyr Val Cys Asn Arg Glu Lys Leu Cys Glu Ser
20 25 30

Leu Gln Ala Val Phe Val Gln Ser Tyr Leu Asp Gln Gly Thr Gln Ile
35 40 45

Phe Leu Asn Asn Ser Ile Glu Lys Ser Gly Trp Leu Phe Ile Gln Leu
50 55 60

Tyr His Ser Phe Val Ser Ser Val Phe Ser Leu Phe Met Ser Arg Thr
65 70 75 80

Ser Ile Asn Gly Leu Leu Gly Arg Gly Ser Met Phe Val Phe Ser Pro
85 90 95

Asp Gln Phe Gln Arg Leu Leu Lys Ile Asn Pro Asp Trp Lys Thr His
100 105 110

Arg Leu Leu Asp Leu Gly Ala Gly Asp Gly Glu Val Thr Lys Ile Met
115 120 125

Ser Pro His Phe Glu Glu Ile Tyr Ala Thr Glu Leu Ser Glu Thr Met
130 135 140

Ile Trp Gln Leu Gln Lys Lys Lys Tyr Arg Val Leu Gly Ile Asn Glu
145 150 155 160

Trp Gln Asn Thr Gly Phe Gln Tyr Asp Val Ile Ser Cys Leu Asn Leu
165 170 175

Leu Asp Arg Cys Asp Gln Pro Leu Thr Leu Leu Lys Asp Ile Arg Ser
180 185 190

Val Leu Glu Pro Thr Arg Gly Arg Val Ile Leu Ala Leu Val Leu Pro
195 200 205

Phe His Pro Tyr Val Glu Asn Val Gly Gly Lys Trp Glu Lys Pro Ser
210 215 220

Glu Ile Leu Glu Ile Lys Gly Gln Asn Trp Glu Glu Gln Val Asn Ser
225 230 235 240

Leu Pro Glu Val Phe Arg Lys Ala Gly Phe Val Ile Glu Ala Phe Thr
245 250 255

Arg Leu Pro Tyr Leu Cys Glu Gly Asp Met Tyr Asn Asp Tyr Tyr Val
260 265 270

Leu Asp Asp Ala Val Phe Val Leu Lys Pro Val
275 280

<210> 181
<211> 125
<212> PRT
<213> Homo sapiens

<400> 181
Pro Ala His Leu Ala Thr Thr Ser Arg Trp Asn Pro Ala Thr Ile Cys
1 5 10 15

Glu Met Gly His Asp Ala Val Gln Trp Arg Val Arg Ala Gly Val Ser
20 25 30

Pro Val Ser Thr Thr Phe Val Thr Asp Val Leu Ser Glu Arg Arg Ser
35 40 45

Leu Pro Ser Leu Thr Cys Leu Lys Arg Pro Glu Pro Glu Ser Ala Leu
50 55 60

Ala Val Ser Leu Arg Pro Ala Pro Gly Gly Ala Ser Leu Leu Pro Arg
65 70 75 80

Trp Gly Arg Phe Pro Gly Pro Arg Gly Leu Arg Cys Arg Leu Pro Leu
85 90 95

His Arg Thr Val Leu Ser Phe Pro His Pro Pro Ser Glu Ala Pro Ala
100 105 110

Tyr Ser Arg Gly Val Asn Lys Gln Met Glu Ala Glu Gly
115 120 125

<210> 182
<211> 65
<212> PRT
<213> Homo sapiens

<400> 182
Leu Pro Ser Leu Thr Cys Leu Lys Arg Pro Glu Pro Glu Ser Ala Leu
1 5 10 15

Ala Val Ser Leu Arg Pro Ala Pro Gly Gly Ala Ser Leu Leu Pro Arg
20 25 30

Trp Gly Arg Phe Pro Gly Pro Arg Gly Leu Arg Cys Arg Leu Pro Leu

35 40 45

His Arg Thr Val Leu Ser Phe Pro His Pro Pro Ser Glu Ala Pro Ala
50 55 60

Tyr
65

<210> 183
<211> 101
<212> PRT
<213> Homo sapiens

<400> 183
Met Ile Gly Leu Gly Ile Gly Cys Ala Gly Gln Arg Asp Gln Ala Pro
1 5 10 15

Pro Tyr Leu Ala Pro Pro Ser Gln Glu Pro Gly Asp Ala Ala Lys Ala
20 25 30

Val Asn Arg Gly Gly Gly Thr Val Gly Ala Ala Gly Ser Arg Gly Trp
35 40 45

Gly Glu Thr Cys Gly His Val Ala Ser Met Ala Pro Ala Cys Gln Ile
50 55 60

Leu Arg Trp Ala Leu Ala Leu Gly Leu Gly Leu Met Phe Glu Val Thr
65 70 75 80

His Ala Phe Arg Ser Gln Gly Arg Gly Ser Leu Val Val Ala Val Gly
85 90 95

Arg Glu Arg Lys Met
100

<210> 184
<211> 646
<212> PRT
<213> Homo sapiens

<400> 184
Met Ile Leu Pro Asp Pro Glu Lys Pro Val Arg Leu Asn Val Lys Tyr
1 5 10 15

Asp Lys Asp Ala Ser Phe Leu Ala Gly Gly Leu Phe Thr Asp Phe Met
20 25 30

Ile Ser Val Ile Ser Glu Asp Asp Ser Ile Ile Lys Asn Ile Asn Pro
35 40 45

Ala Arg Ile Ser Met Lys Met Trp Lys Leu Ser Thr Ser Gly Asn Arg
50 55 60

Pro Pro Ala Asn Ala Glu Thr Phe Ser Cys Asn Lys Ile Lys Asp Asn
65 70 75 80

Asp Lys Glu Asp Gly Cys Phe Tyr Phe Arg Asp Lys Val Ile Pro Asn
85 90 95

Lys Val Gly Thr Tyr Cys Ile Gln Phe Gly Phe Met Met Asp Lys Thr
100 105 110

Asn Ile Leu Asn Ser Glu Gln Val Ile Val Glu Val Leu Pro Asn Gln
115 120 125

Pro Val Lys Leu Val Pro Lys Ile Lys Pro Pro Thr Pro Ala Val Ser
130 135 140

Asn Val Arg Ser Val Ala Ser Arg Thr Leu Val Arg Asp Leu His Leu
145 150 155 160

Ser Ile Thr Asp Asp Tyr Asp Asn His Thr Gly Ile Asp Leu Val Gly
165 170 175

Thr Ile Ile Ala Thr Ile Lys Gly Ser Asn Glu Glu Asp Thr Asp Thr
180 185 190

Pro Leu Phe Ile Gly Lys Val Arg Thr Leu Glu Phe Pro Phe Val Asn
195 200 205

Gly Ser Ala Glu Ile Met Ser Leu Val Leu Ala Glu Ser Ser Pro Gly
210 215 220

Arg Asp Ser Thr Glu Tyr Phe Ile Val Phe Glu Pro Arg Leu Pro Leu
225 230 235 240

Leu Ser Arg Thr Leu Glu Pro Tyr Ile Leu Pro Phe Met Phe Tyr Asn
245 250 255

Asp Val Lys Lys Gln Gln Gln Met Ala Ala Leu Thr Lys Glu Lys Asp
260 265 270

Gln Leu Ser Gln Ser Ile Val Met Tyr Lys Ser Leu Phe Glu Ala Ser
275 280 285

Gln Gln Leu Leu Asn Glu Met Lys Cys Gln Val Glu Glu Ala Arg Leu
290 295 300

Lys Glu Ala Gln Leu Arg Asn Glu Leu Lys Ile His Asn Ile Asp Ile
305 310 315 320

Pro Thr Thr Gln Gln Val Pro His Ile Glu Ala Leu Leu Lys Arg Lys
325 330 335

Leu Ser Glu Gln Glu Glu Leu Lys Lys Lys Pro Arg Arg Ser Cys Thr
340 345 350

Leu Pro Asn Tyr Thr Lys Gly Ser Gly Asp Val Leu Gly Lys Ile Ala
355 360 365

His Leu Ala Gln Ile Glu Asp Asp Arg Ala Ala Met Val Ile Ser Trp
370 375 380

His Leu Ala Ser Asp Met Asp Cys Val Val Thr Leu Thr Thr Asp Ala

385 390 395 400

Ala Arg Arg Ile Tyr Asp Glu Thr Gln Gly Arg Gln Gln Val Leu Pro
405 410 415

Leu Asp Ser Ile Tyr Lys Lys Thr Leu Pro Asp Trp Lys Arg Ser Leu
420 425 430

Pro His Phe Arg Asn Gly Lys Leu Tyr Phe Lys Pro Ile Gly Asp Pro
435 440 445

Val Phe Ala Arg Asp Leu Leu Thr Phe Pro Asp Asn Val Glu His Cys
450 455 460

Glu Thr Val Phe Gly Met Leu Leu Gly Asp Thr Ile Ile Leu Asp Asn
465 470 475 480

Leu Asp Ala Ala Asn His Tyr Arg Lys Glu Val Val Lys Ile Thr His
485 490 495

Cys Pro Thr Leu Leu Thr Arg Asp Gly Asp Arg Ile Arg Ser Asn Gly
500 505 510

Lys Phe Gly Gly Leu Gln Asn Lys Ala Pro Pro Met Asp Lys Leu Arg
515 520 525

Gly Met Val Phe Gly Ala Pro Val Pro Lys Gln Cys Leu Ile Leu Gly
530 535 540

Glu Gln Ile Asp Leu Leu Gln Gln Tyr Arg Ser Ala Val Cys Lys Leu
545 550 555 560

Asp Ser Val Asn Lys Asp Leu Asn Ser Gln Leu Glu Tyr Leu Arg Thr
565 570 575

Pro Asp Met Arg Lys Lys Lys Gln Glu Leu Asp Glu His Glu Lys Asn
580 585 590

Leu Lys Leu Ile Glu Glu Lys Leu Gly Met Thr Pro Ile Arg Lys Cys
595 600 605

Asn Asp Ser Leu Arg His Ser Pro Lys Val Glu Thr Thr Asp Cys Pro
610 615 620

Val Pro Pro Lys Arg Met Arg Arg Glu Ala Thr Arg Gln Asn Arg Ile
625 630 635 640

Ile Thr Lys Thr Asp Val
645

<210> 185
<211> 68
<212> PRT
<213> Homo sapiens

<400> 185
Ala Arg Gly Trp Cys Leu Cys Pro Phe Asp Met Thr Leu Ser Val Leu

1 5 10 15

Gln His Phe Phe Ile Cys Val Leu Leu Ile Leu Leu Leu Asp Thr Asn
20 25 30

Leu Cys Arg Gln Ile Ser Ser His Ser Phe Glu Phe Ser Gly Asn Gln
35 40 45

Pro Leu Val Phe Cys Cys Ile Ser Ser Ile Ser Ala Lys Leu Val Leu
50 55 60

Asp Gln Ala Gly
65

<210> 186
<211> 422
<212> PRT
<213> Homo sapiens

<400> 186
Thr Phe Leu Ser Ala Ser Ile Leu Phe Ala Leu Ala Thr Pro Val Gln
1 5 10 15

Ala Gln Asp Ala Gly Leu Phe Asp Glu Val Val Val Ser Ala Thr Arg
20 25 30

Thr Asn Gln Thr Met Asp Ser Val Ala Ala Ser Val Thr Val Ile Ser
35 40 45

Asp Lys Asp Leu Glu Ser Lys Met Ala Lys Asp Leu Asn Asp Val Phe
50 55 60

Glu Tyr Thr Pro Gly Val Thr Ile Asn Ser Thr Gln Arg Gln Gly Val
65 70 75 80

Gln Ser Ile Asn Ile Arg Gly Met Glu Gly Lys Arg Val Lys Ile Leu
85 90 95

Val Asp Gly Ala Ser Gln Pro Gly Val Phe Asp Gly Gly Pro Tyr Ser
100 105 110

Phe Ile Asn Ser Ser Ala Val Ser Val Asp Pro Asp Met Leu Lys Ser
115 120 125

Val Glu Ile Val Lys Gly Ala Ala Ser Ser Leu His Gly Ser Asp Ala
130 135 140

Ile Gly Gly Val Val Ala Phe Glu Thr Lys Asp Pro Ala Asp Phe Leu
145 150 155 160

Lys Asn Gly Lys Val Phe Gly Gly Gln Ala Lys Leu Ser Tyr Ser Ser
165 170 175

Glu Asp Lys Ser Phe Ser Glu His Val Ala Val Ala Lys Arg Phe Asp
180 185 190

Asn Val Glu Ala Leu Val Ala Tyr Thr Arg Arg Asp Gly Lys Glu Leu

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        195                 200                 205
Gln Asn Phe Ser Lys Ala Pro Tyr Asp Asp Tyr Ser Val Glu Ser Gln
    210                 215                 220
Asp Tyr Val Lys Asn Asp Leu Leu Ile Lys Leu Gln Ser Gln Leu Ser
225                 230                 235                 240
Asp Asp His Arg Leu Glu Phe Leu Gly Glu Val Ile Tyr Asn Gln Asn
                245                 250                 255
Asp Ser Asp Ile Ala Ser Ser Ser Tyr Lys Asn Phe Asn Ser Glu Asp
            260                 265                 270
Thr Thr Lys Gln Ser Arg Leu Gly Leu Lys His Ile Trp Leu Val Asp
        275                 280                 285
Thr Thr Ile Thr Asp Ser Ile Thr Ser Arg Leu Thr Trp Thr Asp Lys
    290                 295                 300
Glu Glu Asn Gly Leu Thr Asn Arg Phe Lys Glu Ala Ser Ala Gly Val
305                 310                 315                 320
Pro Pro Trp Val Pro Pro Asn Gly Asp Asn Gln Gln Lys Lys Asp Tyr
                325                 330                 335
Gln Tyr Thr Glu Asn Lys Ile Glu Leu Glu Thr Gln Phe Asp Lys Glu
            340                 345                 350
Leu Glu Ala His Tyr Leu Val Tyr Gly Leu Ser Tyr Lys Ile Ser Arg
        355                 360                 365
Ile Ser Asn Thr Asn Arg Glu Phe Asn Ser Asp Pro Asn Thr Pro Asp
    370                 375                 380
Lys Ile Tyr Val Tyr Thr Pro Asp Ala Lys Glu Met Ser Phe Gly Leu
385                 390                 395                 400
Phe Leu Gln Asp Glu Ile Ser Ile Leu Asn Asp Lys Leu Val Leu Thr
                405                 410                 415
Pro Gly Val Arg Tyr Asp
            420
```

<210> 187
<211> 438
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (42)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (251)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (340)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (429)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 187
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Thr Leu Leu Ala Met Ala Val Ala Ala Ser Leu Ser Phe Thr Val His
1               5                   10                  15

Ala Glu Thr Ala Ala Asp Ala Ser Thr Leu Asp Thr Val Arg Val Gln
            20                  25                  30

Ala Glu Arg Ala Lys Lys Thr Arg Ser Xaa Asn Gln Asn Val Thr Val
        35                  40                  45

Leu Thr Ala Ala Asp Leu Asp Asn Glu Met Ala Asn Thr Met Glu Glu
    50                  55                  60

Ala Ile Arg Tyr Ile Pro Gly Val Ser Ile Val Asp Met Gly Arg Phe
65                  70                  75                  80

Gly Asp Asn Gly Phe Asn Ile Arg Gly Leu Glu Ser Asp Arg Val Ala
                85                  90                  95

Ile Thr Val Asp Gly Leu Ser Leu Gly Glu Ser Val Glu Thr Ala Arg
            100                 105                 110

Ser Tyr Glu Phe Phe Arg Gly Gly Arg Gly Asp Val Asp Ile Asp Thr
        115                 120                 125

Leu Lys Ser Leu Ala Val Ile Lys Gly Ala Asp Ser Ile Ser Ala Gly
    130                 135                 140

Ser Gly Ala Leu Gly Gly Ala Val Val Phe Thr Thr Lys Asp Pro Ala
145                 150                 155                 160

Asp Tyr Leu Lys Pro Ala Gly Asn Asp Thr His Leu Gly Phe Lys Ala
                165                 170                 175

Gly Tyr Ser Gly Ala Asn Asp Glu Thr Met Gly Thr Leu Thr Phe Ala
            180                 185                 190

Asn Arg Thr Gly Ile Val Glu Ser Met Leu Val Tyr Thr Arg Arg Glu
        195                 200                 205

Gly His Glu Ser Glu Ser Trp Tyr Asp Thr Thr Asn Asp Arg Ile Gly
    210                 215                 220

Val Gly Arg Arg Thr Pro Asp Pro Val Asp Ser Thr Arg Asp Asn Leu
225                 230                 235                 240
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Leu Gly Lys Leu Asp Leu Gln Leu Asp Glu Xaa Asn Thr Leu Gly Phe
245 250 255

Leu Tyr Glu Arg Gly Arg Ala Thr Asn Asp Val Asp Asn Leu Ser Arg
260 265 270

Val Tyr Ala Pro Gly Tyr Leu Ser Arg Lys Gly His Asp Thr Asn Asp
275 280 285

Arg Asp Arg Tyr Gly Val Asn Tyr Gln Trp Arg Ala Asp Thr Ala Leu
290 295 300

Phe Asp Thr Leu Asp Ala Gln Val Asp Arg Gln Val Thr Asp Ser Arg
305 310 315 320

Gly Ile Thr Thr Ile Val Ala Gly Ser Gly Cys Pro Gly Gly Ala Thr
325 330 335

Pro Cys Leu Xaa Ser Glu Asn Arg Ser Thr Lys Gln Thr Leu Asp Arg
340 345 350

Ala Ala Ala Asp Phe Ser Lys Val Phe Ala Thr Ala Gly Ala Arg His
355 360 365

Asp Val Val Tyr Gly Leu Ala Trp Gln Gln Arg Asp Ile Asp Phe Thr
370 375 380

Ala Val Asp Thr Arg Trp Asn Ala Ala Gly Ala Ile Ala Ser Val Glu
385 390 395 400

Ile Asp Pro Arg Gln Val Pro Lys Thr Asp Val Thr Ala Trp Asn Leu
405 410 415

Tyr Leu Arg Asp Ser Val Gln Leu Ala Gly Arg Thr Xaa Asp Leu Ser
420 425 430

Ala Gly Ala Arg Tyr Asp
435

<210> 188
<211> 538
<212> PRT
<213> Homo sapiens

<400> 188
Met Lys Ser Ser Leu Thr Leu Leu Ala Met Ala Val Ala Ala Ser Leu
1 5 10 15

Ser Phe Thr Val His Ala Glu Thr Ala Ala Asp Ala Ser Thr Leu Asp
20 25 30

Thr Val Arg Val Gln Ala Glu Arg Ala Lys Lys Thr Arg Ser Ala Asn
35 40 45

Gln Asn Val Thr Val Leu Thr Ala Ala Asp Leu Asp Asn Glu Met Ala
50 55 60

Asn Thr Met Glu Glu Ala Ile Arg Tyr Ile Pro Gly Val Ser Ile Val
65 70 75 80

Asp Met Gly Arg Phe Gly Asp Asn Gly Phe Asn Ile Arg Gly Leu Glu
85 90 95

Ser Asp Arg Val Ala Ile Thr Val Asp Gly Leu Ser Leu Gly Glu Ser
100 105 110

Val Glu Thr Ala Arg Ser Tyr Glu Phe Phe Arg Gly Gly Arg Gly Asp
115 120 125

Val Asp Ile Asp Thr Leu Lys Ser Leu Ala Val Ile Lys Gly Ala Asp
130 135 140

Ser Ile Ser Ala Gly Ser Gly Ala Leu Gly Gly Ala Val Val Phe Thr
145 150 155 160

Thr Lys Asp Pro Ala Asp Tyr Leu Lys Pro Ala Gly Asn Asp Thr His
165 170 175

Leu Gly Phe Lys Ala Gly Tyr Ser Gly Ala Asn Asp Glu Thr Met Gly
180 185 190

Thr Leu Thr Phe Ala Asn Arg Thr Gly Ile Val Glu Ser Met Leu Val
195 200 205

Tyr Thr Arg Arg Glu Gly His Glu Ser Glu Ser Trp Tyr Asp Thr Thr
210 215 220

Asn Asp Arg Ile Gly Val Gly Arg Arg Thr Pro Asp Pro Val Asp Ser
225 230 235 240

Thr Arg Asp Asn Leu Leu Gly Lys Leu Asp Leu Gln Leu Asp Glu Ala
245 250 255

Asn Thr Leu Gly Phe Leu Tyr Glu Arg Gly Arg Ala Thr Asn Asp Val
260 265 270

Asp Asn Leu Ser Arg Val Tyr Ala Pro Gly Tyr Leu Ser Arg Lys Gly
275 280 285

His Asp Thr Asn Asp Arg Asp Arg Tyr Gly Val Asn Tyr Gln Trp Arg
290 295 300

Ala Asp Thr Ala Leu Phe Asp Thr Leu Asp Ala Gln Val Asp Arg Gln
305 310 315 320

Val Thr Asp Ser Arg Gly Ile Thr Thr Ile Val Ala Gly Ser Gly Cys
325 330 335

Pro Gly Gly Ala Thr Pro Cys Leu Arg Ser Glu Asn Arg Ser Thr Lys
340 345 350

Gln Thr Leu Asp Arg Ala Ala Ala Asp Phe Ser Lys Val Phe Ala Thr
355 360 365

Ala Gly Ala Arg His Asp Val Val Tyr Gly Leu Ala Trp Gln Gln Arg

370 375 380

Asp Ile Asp Phe Thr Ala Val Asp Thr Arg Trp Asn Ala Ala Gly Ala
385 390 395 400

Ile Ala Ser Val Glu Ile Asp Pro Arg Gln Val Pro Lys Thr Asp Val
405 410 415

Thr Ala Trp Asn Leu Tyr Leu Arg Asp Ser Val Gln Leu Leu Asp Glu
420 425 430

Arg Leu Thr Leu Ser Ala Gly Ala Arg Tyr Asp Arg Tyr Asp Tyr Ser
435 440 445

Pro Gln Val Asp Ala Thr Phe Val Asp Arg Thr Gly Thr Val Arg Asp
450 455 460

Val Ser Phe Ala Ser Pro Ser Trp Gln Ala Gly Ala Glu Tyr Arg Phe
465 470 475 480

Leu Pro Asp His Ala Leu Trp Ala Gln Val Gly Arg Gly Phe Arg Ala
485 490 495

Pro Thr Val Ala Asp Met Tyr Ser Pro Thr Ser Ala Thr Gln Val Ile
500 505 510

Asn Ala Gln Asn Gly Gln Pro Leu Leu Leu Asn Asp Thr Val Ser Asn
515 520 525

Pro Asp Leu Asp Ser Glu Lys Ser Leu Asn
530 535

<210> 189
<211> 68
<212> PRT
<213> Homo sapiens

<400> 189
Asp Pro Tyr Arg Val Leu Gly Val Ser Arg Thr Ala Ser Gln Ala Asp
1 5 10 15

Ile Lys Lys Ala Tyr Lys Lys Leu Ala Arg Glu Trp His Pro Asp Lys
20 25 30

Asn Lys Asp Pro Gly Ala Glu Asp Lys Phe Ile Gln Ile Ser Lys Ala
35 40 45

Tyr Glu Ile Leu Ser Asn Glu Glu Lys Arg Ser Asn Tyr Asp Gln Tyr
50 55 60

Gly Asp Ala Gly
65

<210> 190
<211> 86
<212> PRT

<213> Homo sapiens

<400> 190
Lys Tyr Leu Leu His Phe Ser His Tyr Val Asn Glu Val Val Pro Asp
1 5 10 15

Ser Phe Lys Lys Pro Tyr Leu Ile Lys Ile Thr Ser Asp Trp Cys Phe
20 25 30

Ser Cys Ile His Ile Glu Pro Val Trp Lys Glu Val Ile Gln Glu Leu
35 40 45

Glu Glu Leu Gly Val Gly Ile Gly Val Val His Ala Gly Tyr Glu Arg
50 55 60

Arg Leu Ala His His Leu Gly Ala His Ser Thr Pro Ser Ile Leu Gly
65 70 75 80

Ile Ile Asn Gly Lys Ile
85

<210> 191
<211> 71
<212> PRT
<213> Homo sapiens

<400> 191
Val Thr Glu Leu Thr Asp Val Thr Tyr Thr Ser Asn Leu Val Arg Leu
1 5 10 15

Arg Pro Gly His Met Asn Val Val Leu Ile Leu Ser Asn Ser Thr Lys
20 25 30

Thr Ser Leu Leu Gln Lys Phe Ala Leu Glu Val Tyr Thr Phe Thr Gly
35 40 45

Ser Ser Cys Leu His Phe Ser Phe Leu Ser Leu Asp Lys His Arg Glu
50 55 60

Trp Leu Glu Tyr Leu Leu Glu
65 70

<210> 192
<211> 59
<212> PRT
<213> Homo sapiens

<400> 192
Val Ile Gln Ala Arg Gly Met Lys Lys Gln Ile Ile Asp Asp Phe Ile
1 5 10 15

Thr Arg Asn Lys Tyr Leu Leu Ala Ala Arg Leu Thr Ser Gln Lys Leu
20 25 30

Phe His Glu Leu Cys Pro Val Lys Arg Ser His Arg Gln Arg Lys Tyr
35 40 45

Cys Val Val Leu Leu Thr Ala Glu Thr Thr Lys
50 55

<210> 193
<211> 61
<212> PRT
<213> Homo sapiens

<400> 193
Arg Phe Leu Ser Gly Trp Gln Gln Glu Asn Lys Pro His Val Leu Leu
1 5 10 15

Phe Asp Gln Thr Pro Ile Val Pro Leu Leu Tyr Lys Leu Thr Ala Phe
20 25 30

Ala Tyr Lys Asp Tyr Leu Ser Phe Gly Tyr Val Tyr Val Gly Leu Arg
35 40 45

Gly Thr Glu Glu Met Thr Arg Arg Tyr Asn Ile Asn Ile
50 55 60

<210> 194
<211> 44
<212> PRT
<213> Homo sapiens

<400> 194
Ser Asn Ser Thr Lys Thr Ser Leu Leu Gln Lys Phe Ala Leu Glu Val
1 5 10 15

Tyr Thr Phe Thr Gly Ser Ser Cys Leu His Phe Ser Phe Leu Ser Leu
20 25 30

Asp Lys His Arg Glu Trp Leu Glu Tyr Leu Leu Glu
35 40

<210> 195
<211> 78
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (46)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (49)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 195
Leu Ser Pro Arg Leu Glu Cys Arg Gly Ala Ile Leu Ala Tyr Cys Asn
1 5 10 15

Pro Cys Leu Leu Gly Ser Ser Asp Ser Pro Thr Ser Ala Ser Arg Val
20 25 30

Ala Val Ser Thr Gly Met Arg Asp His Val Trp Leu Ile Xaa Val Phe
35 40 45

Xaa Val Glu Met Gly Phe Arg His Val Gly Gln Ala Gly Leu Lys Leu
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Leu Ala Ser Arg Asp Ser Ser Ala Leu Val Ser Gln Ser Ala
65 70 75

<210> 196
<211> 155
<212> PRT
<213> Homo sapiens

<400> 196
Met Leu Ser Leu Phe Ser Arg Ala Tyr Leu Pro Pro Val Phe Leu Phe
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Ile Tyr Leu Leu Ile Leu Arg Arg Ser Leu Thr Leu Ser Pro Arg Leu
20 25 30

Glu Cys Arg Gly Ala Ile Leu Ala Tyr Cys Asn Pro Cys Leu Leu Gly
35 40 45

Ser Ser Asp Ser Pro Thr Ser Ala Ser Arg Val Ala Val Ser Thr Gly
50 55 60

Met Arg Asp His Val Trp Leu Ile Phe Val Phe Leu Val Glu Met Gly
65 70 75 80

Phe Arg His Val Gly Gln Ala Gly Leu Lys Leu Leu Ala Ser Arg Asp
85 90 95

Ser Ser Ala Leu Val Ser Gln Ser Ala Gly Thr Ala Asp Met Ser His
100 105 110

Trp Ala Trp Trp Pro Pro Val Tyr Phe Leu Asn Val Phe Ile Leu Phe
115 120 125

Thr His Phe Ser Ile Trp Leu Phe Gly Ile Thr Glu Leu Ser Phe Ile
130 135 140

Leu Asp Ser Cys Ile Tyr Phe Glu Ser Val Phe
145 150 155

<210> 197
<211> 29
<212> PRT
<213> Homo sapiens

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Gln Val Ser Ser Gly Lys Val Ala His Cys Gly Phe Ile
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Arg Pro His Asp Ser Ala Arg Ala Gly Leu Gln Leu Arg Leu Thr Gln
20 25 30

Thr Gly Gly Gln Pro Pro Ala Pro Gly Pro Ala Pro Arg Thr Gly Pro
35 40 45

Pro Ser Leu Pro Pro Pro Leu Leu Ala Pro Arg Thr Gly Phe Ser Glu
50 55 60

Phe Ser Ser Pro Glu Ala Gly Ala Arg Leu Glu Ala Arg Pro Gly Gly
65 70 75 80

Ser Gly Leu Gly Ser Gln Ile Gly Ala Gly Arg Pro Gly Leu Glu Ser
85 90 95

Gly Ile Gln Pro His Ser Trp His Leu Gln Glu Pro Arg Gly Glu Gly
100 105 110

Asp Leu Gly Trp Thr Ser Thr Ser Gln Gly Trp Ile Leu Ala Pro Glu
115 120 125

Pro Ala Phe Leu Gly Gln Pro Arg Asp Pro Ser His Phe Trp Ala Gln
130 135 140

Leu Gly Pro Ala Trp His Ser Arg Pro Ile Trp Gly Leu Gly Val Ser
145 150 155 160

Pro Gln Leu Ser Asp Ile Arg Thr Pro Phe Gln Ala Cys Ser Phe Thr
165 170 175

Trp Arg Gly Pro Glu Pro His Thr Pro Pro Leu Ser Phe Leu His Pro
180 185 190

Thr Ser Ser Ala Ser Leu Pro Leu Leu Ala Gln Ser Leu Arg Trp Gly
195 200 205

Thr Gln His Leu Thr Gly Gly Pro Ser Ser Gly Ala Val Cys Val Gly
210 215 220

Gly Ala Gly Ala Ala Pro Lys Arg Pro Trp Arg Gln Thr Leu Arg Gly
225 230 235 240

Arg Ser Arg Gly Trp Gln Trp Met Leu Cys Leu Glu Ala Leu Arg Ala

245 250 255

Arg Cys Asp Asp Glu Ala Gln Ala Ala Gly Leu Phe Leu Ala Leu Gln
260 265 270

Leu Arg Arg Thr Arg Asp Phe Leu Leu Leu Cys Pro Pro Ser Pro Ala
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<212> PRT
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20 25 30

Gly Phe Cys Ala Gly Cys Glu Glu Tyr Gly Ser Arg Thr Pro Gly Arg
35 40 45

Trp Gln Arg Leu Gly Gly Gln Arg Ala Gly Val Gln Gly Gly Asn Ser
50 55 60

Gln Val Pro Leu Ala Thr Pro Ala Ala Gly Ser Pro Ala His Pro Pro
65 70 75 80

Gly Ala Trp Met Ser Asn Leu Thr Ala Ile Pro Thr Ser Ser Gln Ala
85 90 95

Trp Arg Trp Arg Pro His Asp Ser Ala Arg Ala Gly Leu Gln Leu Arg
100 105 110

Leu Thr Gln Thr Gly Gly Gln Pro Pro Ala Pro Gly Pro Ala Pro Arg
115 120 125

Thr Gly Pro Pro Ser Leu Pro Pro Pro Leu Leu Ala Pro Arg Thr Gly
130 135 140

Phe Ser Glu Phe Ser Ser Pro Glu Ala Gly Ala Arg Leu Glu Ala Arg
145 150 155 160

Pro Gly Gly Ser Gly Leu Gly Ser Gln Ile Gly Ala Gly Arg Pro Gly
165 170 175

Leu Glu Ser Gly Ile Gln Pro His Ser Trp His Leu Gln Glu Pro Arg
180 185 190

Gly Glu Gly Asp Leu Gly Trp Thr Ser Thr Ser Gln Gly Trp Ile Leu

195 200 205

Ala Pro Glu Pro Ala Phe Leu Gly Gln Pro Arg Asp Pro Ser His Phe
210 215 220

Trp Ala Gln Leu Gly Pro Ala Trp His Ser Arg Pro Ile Trp Gly Leu
225 230 235 240

Gly Val Ser Pro Gln Leu Ser Asp Ile Arg Thr Pro Phe Gln Ala Cys
245 250 255

Ser Phe Thr Trp Arg Gly Pro Glu Pro His Thr Pro Pro Leu Ser Phe
260 265 270

Leu His Pro Thr Ser Ser Ala Ser Leu Pro Leu Leu Ala Gln Ser Leu
275 280 285

Arg Trp Gly Thr Gln His Leu Thr Gly Gly Pro Ser Ser Gly Ala Val
290 295 300

Cys Val Gly Gly Ala Gly Ala Ala Pro Lys Arg Pro Trp Arg Gln Thr
305 310 315 320

Leu Arg Gly Arg Ser Arg Gly Trp Gln Trp Met Leu Cys Leu Glu Ala
325 330 335

Leu Arg Ala Arg Cys Asp Asp Glu Ala Gln Ala Ala Gly Leu Phe Leu
340 345 350

Ala Leu Gln Leu Arg Arg Thr Arg Asp Phe Leu Leu Leu Cys Pro Pro
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Ser Pro Ala Ser Ala Cys Ser Ala Ser Ala Pro His Leu Gly Ala Leu
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385 390 395

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<212> PRT
<213> Homo sapiens

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Pro Ala Pro Ala Leu Trp Val Trp Gly Leu Leu Leu Ser Ser Ser Phe
20 25 30

Gln Thr Leu Leu Pro Ala Phe Pro Lys Pro Pro Ala Leu Asn Leu Gly
35 40 45

Cys Ser Thr Arg Pro Ile Pro Ser Phe Leu Lys Ile
50 55 60

<210> 201
<211> 47
<212> PRT
<213> Homo sapiens

<400> 201
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Leu Leu Phe Phe Pro Ala Val Cys Val Cys Val Cys Val Cys Val Cys
20 25 30

Ala Cys Thr Arg Thr Arg Val Cys Asp Glu Thr Ile Lys Leu Val
35 40 45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30045

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to Internati nal Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
X,P	Database GenBank (GenEmbl); Accession NO: AC069062; Birren et al.; Chromosome 2 clone; 04 June 2000; having 99.8% sequence identity with SEQ ID NO: 11, vector; M13 ,see alignment result 1.	1-7, 21
X	Database GenBank (N_Genseq_36); Accession NO: T24525; Matsubara et al.; "Identifying gene signature..."; 25 September 1996; having 98.5% sequence identity with SEQ ID NO: 11, see alignment result 1.	1-7, 21
X,P	Database GenBank (EST); Accession NO: AW451106; NCI-CGAP; tumor gene index; 17 February 2000; having 99.2% sequence identity with SEQ ID NO: 11; vector: pT7T3D; host cell: DH10B, see alignment result 2.	1-10, 21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 JANUARY 2001

Date of mailing of the international search report

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer
Dorothea Lawrence for
RITA MITRA

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30045

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10, 14, 15 and 21, all in part.

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/30045

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07H 21/04; C07H 21/04; C07H 21/02; C07K 5/00; C07K 14/00; C12Q 1/68; C12N 1/21; C12N 15/63; C12N 15/85, 15/86

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1, 23.5, 24.31; 530/300, 350; 435/6, 69.1, 252.3, 320.1, 325

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

536/23.1, 23.5, 24.31; 530/300, 350; 435/6, 69.1, 252.3, 320.1, 325
514/44

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, STN (Database: Biosis, Embase, Medline, Scisearch, Lifesci, Caplus)

Search terms: secreted protein or peptide, polynucleotide, DNA, RNA, nucleic acid, oligonucleotide, fibulin glycoprotein, placenta or brain or colon or ovary, extracellular matrix, chromosome2

GenBank (Database: GenEmbl, N_Genseq_36, Issued_patents, EST, A_Genseq_36, PIR_65, SwissProt_39, Sptrembl_14)

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Groups 1-80, claim(s)1-10, 14, 15 and 21, all in part, drawn to an isolated nucleic acid of SEQ ID NO X or a peptide of SEQ ID NO NO: Y, wherein X and Y are values that correlate to those listed in Table 1, and correspond to one of the cDNA Clone IDs, respectively. For example,

If group 1 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein X is 11 and Y is 91.

If group 2 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein X is 12 and Y is 92.

Groups 81-160, claim(s) 11, 12, 16 and 23, all in part, each group directed t a peptide of SEQ ID NO; Y, wherein Y correlates t one f those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 81 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein Y is 91.

If group 82 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein Y is 92.

Groups 161-240, claim 13, in part, drawn to an isolated antibody which binds to a protein with SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 161 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein Y is 91.

If group 162 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein Y is 92.

Groups 241-320, claim 17, in part, drawn to a method for preventing, treating or ameliorating an undefined medical condition by administering a polypeptide of SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 241 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein Y is 91.

If group 242 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein Y is 92.

Groups 321-400, claim 17, in part, drawn to a method for preventing, treating or ameliorating an undefined medical condition by administering a polypeptide of SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 321 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein X is 11 and Y is 91.

If group 322 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein X is 12 and Y is 92.

Groups 401-480, claims 18 and 19, in part, drawn to a method of diagnosis of an undefined pathological condition by determining the presence or absence of a mutation in a polynucleotide of SEQ ID NO X, wherein X correlates to one of those listed in Table 1, and corresponds to one of the cDNA Clone IDs, respectively. For examples,

If group 401 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein X is 11.

If group 402 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein X is 12.

Groups 481-561, claim 20, in part, drawn to a method of identifying a binding partner to a polypeptide defined by SEQ ID NO Y, wherein Y correlates to one of those listed in Table 1, and corresponds to one of the cDNA Cl ne IDs, respectively. For examples,

If group 481 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein Y is 91.

If group 482 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein Y is 92.

Groups 562-641, claim 22, in part, drawn to a method of identifying an activity in a biol gical assay by identification f the protein in the supernatant wherein the cell expresses a polypeptide encoded by SEQ ID NO X, wherein X correlates to ne f those listed in Table 1, and corresponds to ne of the cDNA Clone IDs, respectively. For examples,

If group 562 is elected, this correlates to Gene NO 1, cDNA clone ID HWLFJ10 of Table 1, wherein X is 11.

If group 563 is elected, this correlates to Gene NO 2, cDNA clone ID HEEAM62, wherein X is 12.

The inventions listed as Groups 1-641 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The polynucleotides and polypeptides of each invention are unrelated, each to each other. The polynucleotide sequences encode structurally distinct polypeptides. Additionally the claimed methods produce different products and/or different results which are not coextensive and which do not share the same technical feature.